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PRINCIPLES and PRACTICE

of

CHEMOTHERAPY

With Special Reference to the

Specific and General

TREATMENT OF SYPHILIS

By

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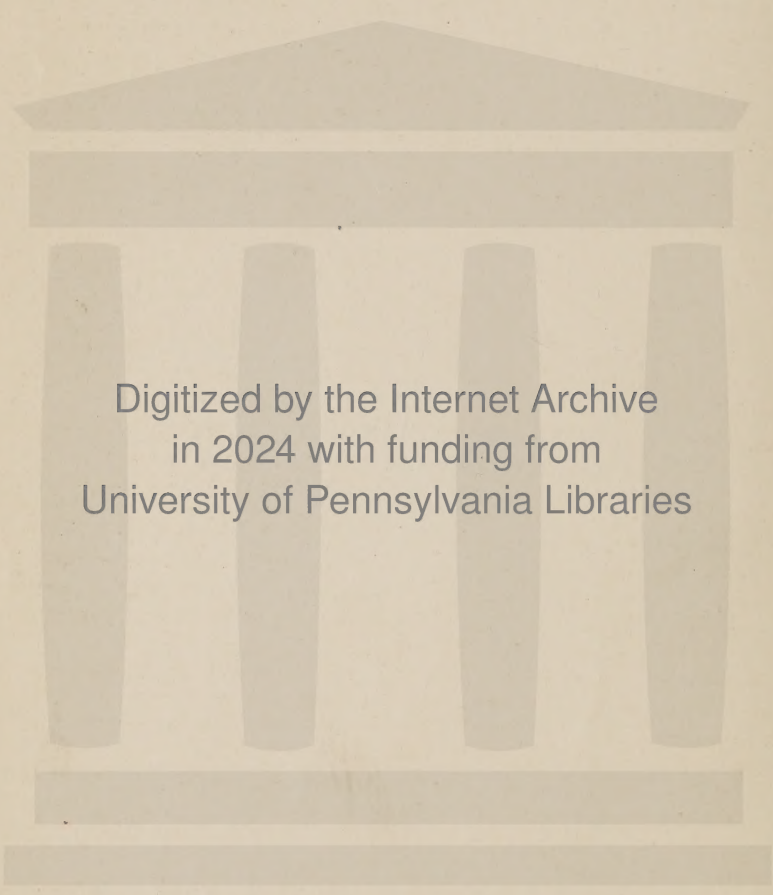
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PREFACE

Two years ago when revising my book on "Infection, Immunity and Specific Therapy" for a third edition, I found it necessary to rewrite entirely the section on chemotherapy and I resolved at that time, with the kind consent of the publishers, to present the subject, including the general and specific treatment of syphilis, in a separate monograph, owing to the very large amount of clinical and laboratory data requiring inclusion and discussion.

I have considered the chemotherapy and general treatment of syphilis in considerable detail, not only because of the great practical importance of the subject, but because I believe that the greatest advance in this new field of therapeutic science has been made in the treatment of syphilis, that its specific and general treatment best serve as examples of the problems and as guides to present and future lines of clinical and laboratory investigation in the chemotherapy of other diseases, and above all, because there is a real and practical need for a treatise devoted principally to the modern treatment of this widespread disease.

Chemotherapy usually conveys the idea of specific therapy, and properly so, but its practical applications are by no means limited to the treatment of syphilis, malaria and a few other infectious diseases, as popular impressions would indicate. It is true that most advance has been made in the treatment of syphilis, relapsing fever, frambesia tropica and other spirochetic infections; in trypanosomiasis, leishmaniasis, schistosomiasis, piroplasmosis, malaria, amebic and other protozoan and metazoan infestments, but some real progress, with practical applications, has been made also in the great and important field of bacterial diseases and especially when chemotherapy is employed for the treatment of local as well as of systemic infections. In other words, the local disinfection of tissues by chemical agents properly belongs to this new field of therapeutic science and greatly broadens its field of application and usefulness.

It is true, however, that some of the original ideas and theories of chemotherapy begin to show themselves inadequate for our expanding knowledge of the processes concerned and require reconstruction, though without either hasty or wholesale rejection of those principles which served Ehrlich and other early workers so well. But the original idea of chemotherapy, tending to shift the focus of attention from the infected host to the invading parasites by producing chemical agents capable of direct destruction of organisms without the coöperation of the host, has been questioned and especially in relation to the disinfection of the tissues in systemic infections, although this simple mechanism may, and probably does, hold good to a large degree for the disinfection of the tissues by local or topical application of parasiticidal agents.

But if the mechanism of chemical disinfection of the tissues involves, as it may, coöperation on the part of the host, sometimes with the production of really new chemical compounds following administration, so likewise that phase of chemotherapy touching upon the physiological and toxic

effects of chemical compounds and drugs for the body cells has proved to be more complex than originally surmised and in large measure has become reunited with its parent subject of pharmacology, to the distinct advantage of therapeutic science.

Naturally, my laboratory and clinical experience have enabled me to lay particular emphasis upon the biological phases of chemotherapy and I have attempted to strike a proper balance between the purely theoretical and practical phases of the subject. My training and experience have not enabled me to delve deeply into the intricate chemical phases, but even were it otherwise, I should have hesitated doing so because of the present chaotic state of our knowledge of this portion of the subject. While this phase has not been neglected or its primary and fundamental importance underestimated, both my laboratory and clinical experience have enabled me to write especially upon the biological aspects and I have embraced the opportunity for including an account of a large amount of hitherto unpublished laboratory and clinical investigations. Necessarily, in a subject like chemotherapy, theoretical discussions must be included and they serve a very useful purpose, but I have endeavored to lay particular emphasis upon the practical phases and applications of chemotherapy in the treatment of disease, which, after all, is the final aim and purpose of this new division of therapeutic science. Indeed, while theory and systematized laboratory investigations have led to results of major importance in practical therapeutics, and must continue to exert the leading and important rôles in the advancement of chemotherapy, yet a reasonably intelligent empiricism is still required and contributes in no small degree toward material advance.

Chemotherapy is properly commanding a large share of attention and effort in medical research, and I have endeavored to make my volume of service to both the investigator and the practicing physician. The subject necessarily touches upon every specialty in the practice of medicine and surgery, not only because it concerns the treatment of infectious diseases in general, but of syphilis in particular, to which every organ and tissue of the human body is vulnerable. As previously stated, the treatment of syphilis has received special and detailed consideration, and while prevailing opinions and methods on the treatment of this disease are numerous and confusing, I have not hesitated to describe with detail methods for the treatment of the different stages of syphilis based upon my laboratory and clinical observations. Few are in better position than I to realize that routine methods for the treatment of syphilis are neither possible nor desirable, yet it is far better to have methods subject to variation to meet individual conditions than to have no methods at all. Throughout the work, the words "arsphenamin" and "neoarsphenamin" are used as synonyms or substitutes for the words "salvarsan" and "neosalvarsan."

The task, while a labor of love, has been by no means a light one. An enormous literature required perusal, sifting and classification, but I hope that I have succeeded in including a sufficient number of references to the important publications to render this monograph of value for reference purposes. I cannot aspire to have included all the references; some, and doubtless important ones, have escaped attention, but I have made no attempt to cover all the available literature on the treatment of syphilis, because this would have materially altered the essential character of the volume and enlarged it too greatly. Neither can I hope to have escaped making mistakes and failing to detect errors in the reading of proofs, but I shall be grateful for having these brought to my attention for correction.

Since the larger part of our knowledge of chemotherapy has been gained from studies on the lower animals, it is not strange that these were early and directly benefited by the practical application of this knowledge in the treatment of some of the protozoan and metazoan diseases to which the lower animals are subject. I have, therefore, included in this work an account of those practical applications of chemotherapy having a direct bearing upon veterinary medicine.

Many illustrations have been included, and it is hoped that they will serve to elucidate the text and to teach, rather than merely to embellish.

In conclusion, I beg to express deep appreciation of the assistance given by Miss Christine Catrevas in reading the proofs and of the unvarying efficiency and courtesy of the publishers.

J. A. K.

Philadelphia, Pa.,
October, 1926.

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PART I

PRINCIPLES OF CHEMOTHERAPY

CHAPTER I

GENERAL PRINCIPLES OF CHEMOTHERAPY

Development of Modern Chemotherapy.—From the earliest times, healers of the sick have sought specific remedies in the form of drugs or methods that would always and unfailingly effect the cure of a certain disease, and this search has been extended for a single remedy that will cure all diseases, regardless of their origin and nature. To this end, throughout the ages experimentation, conscious or otherwise, has gone hand in hand with medicine. Countless substances gathered from the vegetable, animal and mineral kingdoms have been experimented with, but for the most part they have been discarded. Despite this long and persistent search for specifics, until comparatively recent times but three remedies have been found worthy of being regarded as specifics, namely, mercury for syphilis, discovered about 1500, cinchona bark for malaria, discovered by the Indians of Peru and brought to Europe by the Jesuits in the 17th century, and ipecacunha for dysentery, discovered by the natives of Brazil and introduced in Europe at about the same time. In this sense, therefore, chemotherapy is not entirely of recent development.

But modern chemotherapy as a new department in pharmacology and therapeutic science, while doubtless stimulated to some extent by these traditional remedies, has had its origin largely in the work of Ehrlich, who as a medical student was greatly impressed with Heubel's work on lead poisoning. This gave him the basis for the idea "that the ways and means by which drugs are distributed over the body must be of the greatest importance in the rational development of therapeutics." This thought guided him into a valuable series of studies upon the selective affinity of synthetic dyes for blood and other body cells and these in turn quite naturally into a consideration of the selective affinities of chemical agents for parasites which led him to pronounce "*corpora non agunt nisi fixata*" as a basic principle for this new science—that parasites can only be destroyed by those materials for which they have a certain affinity and by which they are fixed or "anchored" by the parasites. This combining affinity of parasites for chemical agents was designated by Ehrlich as *parasitotropy*, but he also knew that they had a combining affinity for the cells of the body designated as *organotropy* and that only those substances can be employed as medicaments in which organotropy and parasitotropy stand in proper proportions.

Doubtless, the discovery of natural and acquired antibodies in diseases and especially the protection and curative activity of diphtheria antitoxin, greatly strengthened Ehrlich's hope and dream of evolving chemical agents capable of reaching and destroying the parasitic causes of disease without injury to the cells of the host. As he stated on one occasion, the antibodies have "no affinity at all for the substances of the body, but they rush exclusively at the parasites. Therefore, in this case organotropy is reduced to zero, parasitotropy is absolute and the antibodies represent magic balls which seek their unerring aim." He never hoped that chemotherapy would ever reach this ideal of specific serum therapy, but that with chemical agents "we shall be obliged to use all our strength to take our aim very accurately in order to hit the parasites as much as possible, the body as little as possible."

But the hopes for serum therapy of various bacterial diseases, aroused by the remarkable success of diphtheria and tetanus antitoxins, did not materialize and when it was realized

that for certain forms of infection, several of which had proved to be infections by protozoa, the body was unable to produce antibodies sufficient for eradicating the disease and that curative immune serums could not be produced, the question arose whether, with the rapidly growing field of synthetic chemistry, man could not match nature's achievements by synthesizing chemical compounds specifically adapted for uniting with and killing the parasites when administered in subtoxic amounts.

Already in 1887 synthetic chemistry had evolved acetanilid (antifebrin) and other substances from coal tar, and while these antipyretics, narcotics and anesthetics were but symptomatic remedies, yet their production opened up a wide field for investigation in chemotherapy. Naturally the synthetic coal tar dyes first attracted the attention of Ehrlich and his colleagues and when Laveran and Mesnil showed how trypanosomiasis could be produced experimentally in mice and rats, the conditions were ripe for therapeutic experimentation in this field, which soon culminated in the discovery by Ehrlich and Shiga of a trypanocidal dye designated as "trypan-red." This was soon followed by the discovery of "trypan-blue" and "Afridol violet" by Mesnil and Nicolle, the latter compound being of special significance because it differed from any previously tested in that its central nucleus was diamino-diphenyl-urea and the forerunner of that valuable colorless, water-soluble salt of a complex sulphonic acid synthesized fifteen years later and known as "Bayer 205" for the treatment of trypanosomiasis.

In the meantime, the parasite of syphilis having been discovered by Schaudinn and the trypanocidal activity of atoxyl, a drug which had been synthesized in the late sixties by an obscure French chemist, Beauchamp, having been discovered by Thomas and Breinl, Ehrlich began his epoch making work with the organic arsenicals, which so impressed him with chemotherapeutic possibilities that he was lead to announce: "*Quod non sanat ferrum, sanat arsenicus; quod non sanat arsenicus, sanat ignis*" (What iron does not cure, arsenic cures; what arsenic does not cure, fire cures) and ending with a complete justification of his faith by the synthesis of salvarsan (arsphenamin), the greatest triumph of modern chemotherapy.

Thus it will be seen that modern chemotherapeutic science is based upon the work and discoveries of many individuals, not only in the realm of synthetic chemistry, but likewise in the fields of parasitology and immunology. No doubt the discovery of the trypanosomes and spirochetes and the means of producing experimental infections on a large and cheap scale was a wonderfully fortunate circumstance, for had the early experiments in chemotherapy been conducted with the bacteria, disappointments would probably have extinguished enthusiasm by a lack of sufficient success and encouragement as subsequent events have amply indicated. To this extent "chance" surely played an important part, just as it did when the discovery of *Spirochæta pallida* induced Wassermann and his colleagues and Detre to try the complement-fixation test in syphilis, in which disease experience has since taught us the phenomenon is more marked and successful than in any other infection.

It is true that some of the theories of Ehrlich are no longer tenable in the light of more recent discoveries and that the underlying principles of chemotherapy are far more complex than he originally conceived them, but yet it is to be remembered that they served him and his colleagues well and proved a wonderful stimulus to chemotherapeutic research, just as his "side chain" theory of immunity stimulated research in the field of immunology. In his opinion, scientific imagination and a healthy optimism were essential for success, and whoever had the opportunity of coming into close contact with him at the time when he worked in small and modest laboratories with few assistants was impressed by the fact that his main activity lay in the mental penetration of problems, as the lack of means permitted him to employ only the simplest technic. But he soon realized that chemotherapeutic discoveries would require a vast amount of work and ample resources. His accomplishments in the field of chemotherapy, which in a certain degree always will be "the sport of experimentation and of chance," were not entirely due to accidental success, but largely the outcome of prolonged, laborious and well systematized laboratory experiments, constituting

the "powder and dynamite" of scientific research and the labors of which can be only fully appreciated by those engaged in similar work.

Definition and Purposes of Chemotherapy.—The term chemotherapy is difficult to define in a brief and concise manner. Of course, chemical agents have been used in the treatment of disease for ages, and following the discoveries of Pasteur, Koch and others of pathogenic bacteria, germicides and antiseptics, attempts were made to disinfect the tissues with mercurial and other compounds, the work of Lister in disinfecting the tissues locally with phenol and related substances meeting with brilliant success. In a general sense, therefore, chemotherapy is the treatment of parasitic diseases with any chemical agent and is almost as old as the art of medicine.

But this definition is not sufficient, because it does not fulfill the principles of the modern definition of chemotherapy, as the term was coined by Ehrlich. It is one thing to *treat* an infectious disease with a chemical agent, but a different one to cure it by destroying the organisms by such means. We may treat a bacterial infection like typhoid fever with small and harmless doses of mercuric chlorid, but we do not cure the disease by such means, because we cannot give sufficient to destroy the bacilli while sparing the tissues and organs of the patient serious damage, which real chemotherapy, the chemotherapy of Ehrlich, seeks to accomplish. Chemotherapy, in a general sense, is simply the treatment of parasitic diseases with chemical agents; chemotherapy, in a modern sense, is the treatment of parasitic diseases by more or less specific disinfection of the tissues by chemical agents without marked or serious toxic effects. The medicament need not be necessarily specific for any one parasite, that is, it need not be strictly mono-parasitotropic; arsphenamin, for example, is destructive not only for *Sp. pallida*, but also for *Sp. pertenuis*, *Sp. obermeieri*, *Sp. gallinarum* and various pathogenic trypanosomes like *Tr. equiperdum*, *Tr. equinum*, etc. Neither need the medicament owe its curative success to a direct parasiticidal activity; it may, for example, accomplish the same end by an indirect means through the production of leukocytosis, stimulating phagocytosis or other immunological defenses. Furthermore, the definition may likewise include the prevention of disease by the destruction of parasites by chemical agents. For example, the prevention of infection in a wound by irrigation with Dakin's solution or some other solution of a chemical substance is, in my opinion, a true example of modern chemotherapy, since it may accomplish the destruction of bacteria without injury to the host.

Chemotherapy may be defined, therefore, as the prevention or treatment of disease by chemical disinfection or inhibition of the parasitic causes without marked or serious toxic effects. The cause may be a bacterial, protozoan or metazoan parasite; prevention or treatment may be the result of a complete destruction of the parasites or a sufficient crippling of their proliferative mechanism (genesistasis) to permit the body cells and fluids to dispose of them. Under ideal conditions the medicament must not harm the body cells or produce toxic reactions at all, but since this ideal is very difficult of actual attainment in the treatment of disease, the requirements may be made less stringent and to read that these effects shall not be of a marked or serious character. The chemical agent need not be necessarily an artificial one produced by synthesis; it may be a natural medicament like quinin of cinchona bark or emetin of epicacuanha. The science of chemotherapy, as defined by Ehrlich, "treats of the action and mechanism of the effects of chemical substances upon cells and especially of drugs upon disease producing organisms." The subject includes, therefore, a study of both organotropic and parasitotropic actions of chemical agents and drugs.

In plain language, the science of chemotherapy constitutes a search for specifics in the treatment of all diseases caused by parasites. The parasites may be so small as to fall into the class of the filterable viruses or as large as the beef tape worm (*Tænia saginata*). They may be on the skin or a mucous membrane and vulnerable to destruction by the direct application or administration of the medicinal agent, or they may be in blood and lymph or entrenched within cells in the depths of an internal organ and approachable only by the enteral or parenteral administration of the medicament. The one essential is that the parasites shall be killed or so crippled that the natural body defenses can complete this task, by doses or amounts of the chemical agent as shall not injure the body cells at all or but to a slight and never serious or fatal extent. When chemotherapy is looked upon in this manner, but yet in the strict modern sense of the term, it is a broad subject intimately concerned with the destruction of parasites by chemical agents and drugs ranging all the way from disinfection of the skin before operation and the destruction of hookworms in the duodenum by carbon tetrachlorid to the routing out of *Sp. pallida* in the aorta of a syphilitic by arsphenamin or one of its congeners.

Parasitotropism and Organotropism in Relation to Chemotherapy.—It will be observed, therefore, that a special and selective affinity or combining power of the chemical agent or drug for the parasite is the predominant thought in modern chemotherapy. As previously stated, Ehrlich designated this state as *parasitotropy* or *parasitotropism* and it is pertinent to inquire into our knowledge concerning the nature and mechanism of this phenomenon. Parasitotropism may be selective affinity of a chemical agent for the protoplasm of a single and particular species of parasite, designated as *mono-parasitotropism* or the affinity may be for several species, designated as *poly-parasitotropism.* Up to the present time, a strictly mono-parasitotropic substance is unknown; at least, I do not know of any chemical agent or drug that is not capable of destroying more than one species of parasite, although its effects may be limited to parasites closely related biologically. Arsphenamin and its congeners, for example, possess a parasitotropism not only for *Sp. pallida,* but also for *Sp. pertenuis*, *Sp. recurrentis* and even for various trypanosomes; quinin is well known as a general protoplasmic poison and the dyes possess a parasitotropism for many different bacteria, etc.

Parasitotropism is not necessarily synonymous with parasiticidal activity. In other words, it is possible for a chemical compound to possess an affinity for the protoplasm of a parasite and to enter into combination with it without necessarily killing the parasite, as, for example, in the case of some of the dyes; indeed, the very reverse may occur and the virulence and activities of the parasites undergo a temporary increase, as in the production of Jarisch-Herxheimer reactions in syphilis by the administration of mercury or arsphenamin and the increased numbers and activity of trypanosomes in the blood of patients with African "sleeping sickness" following the administration of "Bayer 205." However, the parasitotropic activity of a chemical compound usually bears an important and primary relationship to parasiticidal and curative activity, although a compound may still prove therapeutically effective without necessarily involving the questions of parasitotropism and parasiticidal activity or inhibition.

Organotropism on the other hand, refers to the selective affinity of chemical compounds and drugs for the blood, lymph and fixed tissues of the body. Naturally, it bears a very important relation to the pharmacological action of drugs and to toxicology. Instances of selective activity of drugs like strychinin, digitalis, morphin, etc., are so numerous and well

known, that it is not necessary to discuss them here in relation to chemotherapy. It may be stated, however, that the selective effect of drugs upon body cells does not necessarily require that they shall enter into chemical union with the protoplasm of the involved cells, but the general principle holds good that the mechanism and nature of parasitotropism and organotropism are very probably identical. When further information is gained, we shall probably learn that the mechanism of both is exceedingly complex, although fundamentally the same, and involving the questions of chemical or physicochemical interactions between parasite and body cells and the chemical agent or drug.

Chemotherapeutic Index.—In order to express the important relation between organotropism and parasitotropism, Ehrlich coined the phrase *chemotherapeutic index* for expressing the relation between toxicity of a compound for the body (organotropism) and toxicity for the parasites (parasitotropism). The ideal and aim of chemotherapy is to evolve compounds with a minimum of organotropism and a maximum of parasitropism or disinfectant activity. As I shall discuss in the succeeding chapter, toxicity or organotropic tests are commonly conducted by administering compounds to the lower animals according to body weight and expressing the results in terms of the smallest amounts causing immediate death or death in twenty-four hours (*dosis lethalis*) or according to the largest amounts per body weight borne with or without symptoms and histological changes for an arbitrary period, like ten days, and designated as the *maximal tolerated dose.*

The parasitropic, curative or disinfectant effects of the compound are then determined by administering it to animals infected with the chosen parasite and the results expressed in terms of the smallest amounts per kilogram of body weight capable of sterilizing completely the blood and fixed tissues. This may be designated as the minimal curative dose (*dosis curativa*) and the whole expressed in the form of an equation as follows:

$$\frac{\text{Maximal Tolerated Dose per Kilogram}}{\text{Minimal Curative Dose per Kilogram}} = \text{Chemotherapeutic Index}$$

A concrete example may better explain this subject. Arsphenamin (disodium salt) by intravenous injection is generally borne by white rats in dose of 0.120 gm. per kilogram for an indefinite period, while 0.140 gm. may prove fatal in a few days; the former, therefore, is approximately the maximal tolerated dose. When administered to rats infected with *Tr. equiperdum*, the smallest dose per kilogram capable of completely eradicating the infection may be 0.004 gm., the minimal curative dose. The results, therefore, are expressed as follows:

$$\frac{0.120}{0.004} = 30 \text{ (Chemotherapeutic Index)}$$

This means that the single minimal curative dose was 30 times less than the single maximum tolerated dose. Various minor modifications of this method have been described by different authors, but the foregoing illustrates the basic principles underlying the determination of the chemotherapeutic index.

The chemotherapeutic index of a compound is subject, however, to wide variations and fluctuations according to the test animal and particular strain of organism employed. In the first place, the toxicity of a compound varies in animals of different species, the mouse, for example, being able to tolerate

larger doses of most compounds than the rat, and the rat more than the dog. Off hand, it would be supposed that a strain of trypanosomes, say *Tr. equiperdum*, would be equally susceptible to a drug like arsphenamin in any susceptible animal, but I suspect that this is not the case and in my experience the organisms floating in the blood of the mouse are sometimes more vulnerable to compounds than those in the blood of the rat and in either animal they are more vulnerable than in the blood or tissues of the guinea-pig and rabbit. Why this should be so has not been adequately explained, but in a general manner the chemotherapeutic index of a compound for pathogenic trypanosomes is apt to be higher in the mouse than in the rat and higher in both of these than in the guinea-pig or rabbit.

Furthermore, the chemotherapeutic index varies among different strains of the same organism. For example, it is lower against *Tr. brucei* than against *Tr. equiperdum*, which may be due to the greater virulence of the former and consequently the larger doses required for the protection and cure of the inoculated animal. But this is not the whole story, since *Tr. lewisi*, but feebly virulent at best, is usually more difficult to kill than the virulent trypanosomes and consequently is apt to give the poorest chemotherapeutic index of all.

There is, therefore, a curious and intimate relationship between toxicity for the host and toxicity for the parasite as expressed in the chemotherapeutic index. In all probability, the metabolization of the compound following administration influences both properties. But for some reason a compound may be less toxic for one animal than for another; likewise less toxic for one strain of a parasite than for another strain of the same species. In a general manner, if a compound has slight avidity for the cells of the host, apparently more is left over, as it were, for the destruction of parasites and yielding thereby a high chemotherapeutic index. If the chemical agent has a strong avidity for the parasites and many parasites are present, the body cells may be spared accordingly, suggesting that the greater tolerance of syphilitics for the arsenicals and mercurials than non-syphilitic individuals may be due to some such mechanism and relationship.

Parasitotropism in Relation to Leads in Chemotherapy.—Parasitotropism is of fundamental importance in relation to "leads" or the discovery of "optimum centers" (Ehrlich) in chemotherapeutic investigations. By this is meant the discovery of a particular kind or group of compounds possessing a selectively high disinfecting effect for a certain parasite *in vitro* and *in vivo*, which by further modifications of the molecule may have its parasitotropism and organotropism so brought into a workable range that the compound may be safely used in the practical treatment of disease. This is the very life and soul of chemotherapeutic research, but unfortunately we know so little of the fundamental laws and principles of parasitotropism that the search for "leads" is almost purely one of guess work and actual experiment. In other words, most leads are furnished by empiric clinical experience indicating that a certain class of compounds appears to influence an infection in a particularly selective manner or by actually carrying out an extensive and laborious series of tests with a certain organism and different compounds to determine which, if any, displays a sufficiently high selective parasitotropism to warrant adopting it as a compound or group worthy of special development. As stated by Ehrlich in his Harben lectures, "it must be the goal of our experiments to search, by systematic chemical studies, for the most suitable substances, and thus to discover the 'optimum centers.' This problem is by no means an easy one, since according to my experience of many years, which agrees entirely with that of Mesnil, different animal

species have different optima, and therefore the work must be specially done for each species of animals and each kind of trypanosomes."

As will be discussed shortly, I believe parasitotropism is purely a matter of chemistry and until we know more about the chemistry of the protoplasm of parasites and of chemical constitution of our compounds in relation thereto, we shall be working largely in the dark groping for leads on the basis of actual trial and experiment instead of on the basis of concrete knowledge of the underlying principles and laws. There is a great need, therefore, for more research upon the fundamental principles of chemotherapy and especially upon this subject of chemical constitution of both parasites and compounds in relation to parasitotropism and leads.

Parasitotropism and Organotropism in Relation to Carriers of Parasiticidal Agents and Vital Staining.—Of further interest and importance in relation to this subject of the specific parasitotropism and organotropism of chemical agents in therapeutic science, is the possibility of employing substances not of themselves parasitocidal but possessing a high degree of parasitotropism, as carriers or vehicles for transporting parasiticidal agents to organisms in the tissues. As von Wassermann said of eosin-selenium mixtures in the treatment of experimental cancers, the dye may act as a "railroad" for conveying the selenium to the cells, although it is not of itself cytotoxic. This idea has long held sway in chemotherapeutic research and especially in relation to the dyes, which may not be parasiticidal of themselves but yet, possibly, capable of sneaking a destroyer into the protoplasm of parasites by utilizing a dye possessing a high degree of parasitropism with low organotropism and attaching to it a more active parasiticidal agent. For local use, however, the dye need not necessarily possess a selective parasitotropism; it may only be capable like fluorescein, of staining organisms and other cells in a nonselective manner, but yet serve as a useful carrier of a potent parasiticidal agent, as the mercury of mercurochrome.

While the dyes may serve a very useful purpose in this regard for aiding the deeper penetration of parasiticidal agents when directly applied to the skin and mucous membranes, they have not so far been successfully utilized for the chemotherapy of systemic infections by the internal or intravenous administration of compounds. This subject involves the question of vital staining which is far more complicated and perplexing than the mere staining of cells by local application and involves more intimately the subject of selective parasitotropism. It is, however, a matter of great importance and especially in relation to the chemotherapy of tuberculosis and other bacterial infections where a great stumbling block to success appears to be a lack of penetration of chemical disinfectants for localized tissue infections even though the disinfectant is present in the blood in parasiticidal concentrations. Indeed, Ehrlich began his work in chemotherapy from the standpoint of the behavior of dyes in the body, so-called *vital staining*, and with Herter expressed the opinion that "it will always be of great interest to produce dyes in which a strongly acting residue is contained, as possibly, in a part of these derivatives, a toxic effect may be produced with the possibility of studying the connection between distribution and effect by means of biological and microscopical investigations." In Ehrlich's own words, chemotherapy in the beginning was essentially a matter of dye therapy, since the quality of a substance to penetrate, locate and become fixed in certain cells and tissues would appear to be of fundamental importance; in his own work he classified the dyes as neutotropic, lipotropic or polytropic, according to their fixation mainly by the nervous system, the fats or the tissues in general of the body.

Needless to state, a large amount of investigation has been devoted to the mechanism of vital staining, but unfortunately our knowledge is still very imperfect although it may be stated that the phenomenon is largely one of colloidal chemistry in which the physico-chemical state of the solutions is of fundamental importance; for example, true solutions apparently do not stain vitally, whereas the so-called hydrophilic colloids possess this property in greatest degree.

Trypan-red and trypan-blue are readily soluble in water and when injected into the lower animals are taken up by special connective tissue cells called by Goldmann[1] "pyrrhol cells," which are so numerous and so filled with deeply colored granules that the connective tissue of the body appears deeply stained. Lewis[2] found that trypan-red penterates to the center of fully formed tubercles in rabbits and DeWitt[3] observed that both dyes penetrated into even the necrotic centers of large tubercles in the spleen and liver of guinea-pigs within a few hours after injection, but unfortunately the tubercle bacilli themselves were stained poorly or not at all. Furthermore, as I shall discuss with more detail in Chapter V, these and other dyes are almost totally lacking in bactericidal properties for tubercle bacilli both *in vitro* and *in vivo*, but since they penetrate tubercles so well it was thought that they may act as vehicles for carrying more potent substances in the form of combination compounds of silver, gold, copper, mercury, iodin, phenol, etc., discussed at length in Chapter III. Suffice it to state at this time, only a few of the mercury compounds have appeared to influence the disease in guinea-pigs, as indicated by a somewhat longer life with less involvement of the tissues.

It would appear, therefore, that dyes capable of merely staining the tissues of an infected area are insufficient for serving as vehicles for bactericidal substances; to be useful in this connection they should possess a selective staining and parasitotropism for the organisms themselves in these tissues and it is possible that combinations with gentian-violet, fuchsin and other dyes may be ultimately produced for the chemotherapy of staphylococcus, streptococcus and other infections. However, if combinations of this sort were found capable of laying down bactericidal substances only in the tissues of a tubercle or other inflammatory exudate, it is reasonable to hope that such may raise the bactericidal properties of the lymph and cells to therapeutic concentrations capable at least of restraining a further extension of the infection.

Sternberg[4] has suggested combining bactericidal substances with tuberculin for the purpose of employing the latter as a vehicle for the selective transmission of bactericidal substances to tubercles, but in all probability such compounds would likewise fail in the sense of failing to convey the disinfectant to the bacilli themselves. It may be, however, that the production of mild focal tuberculin reactions in combination with chemotherapy and especially the intravenous administration of gold and mercury compounds may be of aid by reason of the hyperemia and serous exudation facilitating the penetration of tubercles with bactericidal agents. Furthermore, this whole question of selective parasitropism of dyes and other compounds is influenced by the question of their solubility reactions and other physico-chemical properties, which I shall discuss shortly in relation to the matter of penetrability of tissues and organisms by chemotherapeutic agents.

Theory of Chemoreceptors in Relation to Parasitotropism and Organotropism.—Naturally, the mechanism of parasitotropism and organotropism is of fundamental importance in chemotherapy and it is just here that our ignorance of this subject hinders and delays progress in therapeutic science

and places the subject so largely at the mercy of "chance" in its development. There is a greater need, therefore, for more investigation along these fundamental lines with the hope that accumulated data and experience will gradually establish the nature and mechanism of the selective affinity of chemical agents for parasites (parasitotropism) and for the body cells (organotropism) and the relation between these properties and the chemical constitution of the compounds.

In searching for a working theory and hypothesis, it was but natural for Ehrlich to swing his view of nutri-receptors and the side-chain theory of immunity into the arena of chemotherapy with the result that a similar theory of receptors was advanced in explanation of parasitotropism and organotropism. As stated in his Harben lectures: "I also wish to lay special stress upon my view that the drugs are attracted by and bound to the protoplasm molecule by certain atom groupings. I am inclined to look upon this as somewhat analogous to the binding of the toxins and of similar protein bodies. Yet on the other hand, there are fundamental differences between the two. For, as I have always insisted, the mode of binding the toxins is peculiar in so far as it is the result of a certain kind of assimilation which obviously consists in processes of a more or less synthetic nature. These toxin-receptors which produce immunity are bodies of a more independent character, and appear to be especially destined for purposes of assimilation. This high degree of independence is evidenced by the fact that, in conformity with my side-chain theory, these receptors are very easily reproduced by the cell in excessive numbers, and after being separated from the cell, find their way into the blood.

"I have now formed the opinion that in like manner a part of the chemically defined substances is attached to the cell by groups corresponding to these receptors; these atom groupings I will distinguish from the toxin receptors by the name of '*chemo-receptors*.' This view is more particularly supported by the fact that an atoxyl-fast strain of trypanosomes is also resistant to a number of substances related to atoxyl, but otherwise showing widely different chemical characteristics. Evidently, therefore, the arsenic acid radical here represents the point of attack which is common to this series of substances. It is the arsenical radical as such, which is bound by the chemo-receptors. These chemo-receptors must, however, be regarded as of a simpler structure than the toxin-receptors. They do not show a similar degree of independence; they cannot therefore, become increased in chronic intoxications, and since they are sessile, they cannot be thrown off into the blood. The number of such chemo-receptors for poisons which a trypanosome cell possesses, represents the number of points of attack. By means of the resistant strains we can count off, one by one, these groups that are open to attack."

According to this theory, therefore, the matter of whether or not a chemical agent displays selective affinity for parasites or body tissues is determined by the presence or absence in these cells of special receptors for the compounds, that is, upon the matter of distribution of specific chemoreceptors, in exactly the same manner that the nutrition of the cells depends upon the presence of receptors for nutriment (nutri-receptors) and that the action of bacterial and other toxins for cells depends upon the presence of special receptors for their anchoring, before they may enter into relationship with the protoplasm. Ehrlich suggested, as quoted above, that these *chemoreceptors* may probably possess a less complex structure, similar to the receptors of the first order of his side-chain theory for simple toxins, that they are more firmly attached to the cell, and that, accordingly, they are less readily cast off, thus explaining why crystalline chemical substances are, as a general rule, incapable of eliciting the production of antibodies. This theory was based upon the discovery that trypanosomes and spirochetes may develop a state of "resistance" or "fastness" to a chemical agent or drug, which Ehrlich thought may be due to a modification of these receptors and which I shall discuss shortly with more detail. Ehrlich also sought to explain the effects of "combination therapy" on the same basis, that is, the possibility of attacking parasites by a combination of chemical agents for which the organisms possess suitable chemoreceptors to effect the union, believing that in this manner the maximum of parasiticidal effect is to be gained with the minimum of injury to the host.

According to this theory, chemical agents and drugs cannot act upon either parasites or body cells unless they are first fixed by suitable chemoreceptors (*corpora non agunt nisi fixata*). Therefore, when arsenic is administered, it is "fixed" by the arseno-receptors of certain cells; mercury in turn is "fixed" by other receptors and so on through an interminable list. Ehrlich thought that the basic principle of chemotherapy was the possibility of producing chemical substances carrying side-arms capable of being fixed by the receptors of parasites to a much greater extent than by the body cells. Presumably it is not necessary that the whole molecule of a chemical agent possess a combining affinity for certain receptors; if one or more atom groups become attached, it is presumed that it carries with it the remainder of the molecule. Moreover, that atom group that is anchored or is responsible for the anchorage of the entire molecule, need not possess any of the properties of the entire molecule or of any part thereof.

Needless to state any theory advanced by the father of modern chemotherapy is deserving of the closest analysis and this theory has at least the merit of having stimulated considerable work on the nature of "drug fastness." But just as his side-chain theory of immunity has been accepted too readily and in the spirit of established fact to the detriment of immunological research on the fundamental principles of immunity, so the theory of chemoreceptors must be remembered as only a theory without permitting it to discourage efforts towards placing parasitropism and organotropism on a purely chemical or physico-chemical basis subject to natural laws. This involves the chemistry of both the body cells and parasites on the one hand, with the chemical constitution of the disinfectant on the other, and it may be now profitable to enquire more closely into these subjects in relation to the nature and mechanism of parasitotropism and organotropism.

Chemical Constitution of Compounds in Relation to Parasitotropism and Organotropism.—Undoubtedly alterations in the chemical constitution of compounds may profoundly influence their toxicity for both host and parasites, but in spite of a great deal of investigation in this field with different classes of compounds and especially those of arsenic, mercury and the dyes, it is still impossible to formulate laws or to synthesize new compounds, the parasitotropism and organotropism of which may be predicted with certainty by their chemical constitution. For this reason, biological experimentation, that is, toxicity and parasiticidal tests, continues to be an integral and important phase of chemotherapeutic investigation and, indeed, owing to the sharp limitations of these procedures, chemotherapy is still largely on an empiric basis.

In succeeding chapters upon the dyes, arsenical, mercurial, hexamethylamin and other compounds, I shall pass in review data bearing upon the relation of chemical constitution to organotropism and parasitotropism; naturally most information has been gained with the arsenicals, but the mass of data is too voluminous to permit of a detailed discussion in this place. Unfortunately, many investigators, probably the majority, have not systematized their work or stuck long enough to any one group of compounds to permit them to study these fundamental relations, a feverish desire to evolve remedies of striking properties quite generally preventing the less spectacular, but nevertheless highly desirable, systematic studies devoted to a painstaking and persistent study of chemical constitution in relation to toxicity and parasiticidal activity and employing the simplest biological methods in order to render all tests strictly comparative with the minimum of experimental error, which will permit the influence of minor alterations in chemical constitution to be properly evaluated.

Even when systematic studies have indicated that the substitution of this or that molecular group renders the compound more or less toxic or parasiticidal, the mechanism of the alteration is not generally elicited, because both of these properties are subject to profound changes by various physical

and physico-chemical agencies aside from the mere matter of direct chemical combination of the compound with the protoplasm of either parasites or body cells. The very magnitude of the subject, the numerous factors to be kept in mind and subjected to study, the frequency of paradoxical and totally unexpected results, contribute toward rendering disinfection in relation to chemical constitution one of the most perplexing of studies, demanding on the part of the investigator an unusual degree of tenacity and the courage to work for years in the face of disappointments. But, as long as the work is arranged on a systematic and orderly basis with accurate and acceptable methods, it cannot fail to be of value, even though it does not result in the production of a remedy of practical application.

In work of this kind the important point is to chose a class of compounds of promise for the treatment of a certain infection, just as Ehrlich concentrated upon the organic arsenicals in relation to the treatment of experimental spirilloses and trypanosomiases. When it was found advisable to reduce the toxicity of arsphenamin, systematic investigations indicated that the introduction of the formaldehyd residue reduced toxicity, just as the introduction of an acetyl (CH_3CO) residue reduced the toxicity of phenetidin and other anilin derivatives. In neoarsphenamin, for example, a condensation product of arsphenamin with sodium formaldehyd sulphoxylate, with complete substitution of one amino-group and partial substitution of the second, both toxicity for the host and for spirochetes is reduced, but the former in greater ratio than the latter; in sulpharsphenamin, both amino-groups are apparently closed with a further reduction in toxicity and especially of pain and irritation upon subcutaneous injection. In the way of additional examples of the influence of molecular substitutions in the organic arsenicals, I may briefly allude to the results observed by Fourneau and his colleagues[5] in their extensive studies with the phenylarsenic acids. The animo-group placed in the para- or meta-position always increased slightly both spirocheticidal and trypanocidal activities with a reduction in toxicity, but these effects were not observed when the animo-group was placed in the ortho-position. The placing of a second amino-group near the amino-group in the para-position still further reduced toxicity, while a third amino-group increased toxicity and diminished therapeutic activity. The introduction of the phenol group OH increased the parasiticidal activity of phenyl arsenic acid to an even greater extent than the substitution of an amino-group, but the position of the OH group was found to be less important. In compound "189" of their series, blocking of the amino-group always reduced trypanocidal activity, no matter what was the nature of the acid rest. Acetylation of the amino-group of this compound, however, reduced spirocheticidal properties but slightly, as shown by the effects observed with compound "190" or "stovarsol." Blocking of the amino-functions of atoxyl gave variable results according to the acid rest: a formic acid rest, for example, increased toxicity and especially for the nervous system while an amino-benzoic rest diminished these effects, etc. It was on the basis of such studies that Fourneau formulated his opinion that the so-called neurotropism of pentavalent arsenicals observed by Ehrlich and others, were due to the presence of impurities, a subject which I will discuss with more detail in Chapter XLVI.

As so clearly shown by Ehrlich and his associates, the valence of the organic arsenicals, therefore, bears a very important relationship to both toxicity for the host and parasiticidal activity, valence being the energy capacity of an element expressed in numerical terms, hydrogen being selected as a basis for its measurement and taken as the unit, namely, 1. When an atom of an element requires only one hydrogen to form a chemical compound, the element is considered univalent. Elements with greater energy capacity are bivalent, trivalent, etc; moreover, they may exist in two or more conditions. For example, iron is both bivalent and trivalent; mercury, univalent and bivalent.

Arsenic belongs to the group of pentavalent elements. In pentavalent condition, all its energy is bound, the capacity for further reaction being greatly diminished. On the other hand, in trivalent condition it is unsaturated, the potential reactivity being much greater.

The chemotherapeutic investigations carried out by Ehrlich and his associates have developed the fact that arsenic in the trivalent condition is a more powerful trypanocide and spirocheticide than arsenic in the penta-

valent condition. There would, in this connection, appear to be established a relationship between the energy capacity of the compound and its destructive influence on the parasites in question. This important observation and, moreover, the fact that chemical elements must be linked to the nuclear carbon in order to exert their fullest effect on the pathogenic microörganisms, are the two most important advancements in modern chemotherapy. This, however, is probably not the whole story, if Voegtlin and his associates[6] are correct in their claim that the difference in toxicity and trypanocidal activity of pentavalent and trivalent arsenic is due to the fact that the pentavalent forms must be reduced to trivalent oxids and that the elimination of pentavalent arsenic is ordinarily so rapid that only a small proportion can be changed into these more toxic substances.

As previously stated, a complete discussion of these interesting and highly important questions would lead me too far afield at present, because each group of compounds introduces special features more or less peculiar to its class. With compounds of mercury, silver and similar heavy metals, for example, I believe that the matter of ionic dissociation bears the most important relation to both toxicity for the host and toxicity for the parasites. Furthermore, the chemical constitution of the protoplasm of body cells and parasites may influence in varying degree the question of chemical or physicochemical interaction between a chemical compound or drug with cells, a subject which deservedly commands a great deal of interest.

While drugs of similar chemical constitution may possess similar biological properties, yet definite changes in the molecule often produce profound alterations in toxicity for body cells and parasites. Certainly the action of chemical compounds is not determined so much by the elementary composition of the substance, as by the manner in which the elements are combined; although with the compounds of heavy metals like mercury, bismuth, gold, copper, tin, etc., I believe that biologic actions are largely influenced by the degree and rapidity of ionic dissociation and that chemical constitution influences toxicity for both host and parasites largely by the looseness or tightness, as it were, of the elementary metal in the compound.

Changes in chemical constitution may result in changes in toxicity of a drug for both host and parasites by influencing either chemical combination with these cells or by altering the physical properties and actions of the drugs. Minor changes may not materially alter these biological properties, although these are sometimes sufficient for evading patent rights and trade marks and resulting in the needless multiplication of drugs for commercial purposes. But attempts to construct a general theory of biological action in relation to chemical constitution have so far failed, although a good deal of useful information has been gained in relation to the dyes, arsenicals, cinchonics, mercurials, etc., which I will review and discuss in later chapters devoted to these and other compounds of chemotherapeutic interest.

Chemistry of Parasites and Body Cells in Relation to Parasitotropism and Organotropism.—Unfortunately, there is but very little data available bearing upon what I believe is an important subject in chemotherapy, namely, the affinity of chemical compounds for the protoplasm of parasites and body cells as influenced by the chemical constitution of the latter. Ehrlich has always insisted that the colloidal nature of protoplasm was no argument against the acceptance of his theory of chemoreceptors, since colloids possess certain groupings of atoms which render them capable of reactions of a synthetic nature; but the question of a chemical combination of arsenic with a protoplasmic constituent as influencing organotropism and parasitotropism did not seriously engage his attention, although Meyer and

Gottlieb had suggested that arsenic causes its toxic effect by reacting with a substance which is present in protoplasm in very small amounts. In this connection, the recent work of Voegtlin, Dyer and Leonard[7] upon the union of arsenic with the sulphydryl (SH) compounds of body cells and trypanosomes is highly suggestive and an important attempt to interpret the action of the arsenicals in terms of known biochemical facts. I will describe this work with more detail in Chapter XII, but here it may be stated that, since Heffter[8] demonstrated that the tissues contain a sulphydryl compound held responsible for its reducing properties and since Hopkins[9] succeeded in isolating from yeast and mammalian tissues one such compound, reduced glutathione, of importance in relation to hydrolytic oxidation-reduction processes of the cell, Voegtlin and his associates have brought forward experimental data suggesting that the trivalent oxids of arsenic, with "arsenoxid" as the type, are specific poisons for the SH group of glutathione and possibly other SH compounds in protoplasm with a consequent suppression of vital functions which may explain the toxic and therapeutic effects of the organic arsenicals like arsphenamin and its congeners.

Since both parasites and body cells contain SH compounds, it is necessary to explain the difference in susceptibility of different types of cells to arsenic; as stated by Voegtlin and his associates:

"It might be reasonably assumed that trypanosomes are more easily killed than rats, for the reason that the former contain and are dependent on a smaller absolute amount of SH compounds than the vital organs of the higher animals. Exposure to a certain concentration of arsenoxid would therefore kill trypanosomes, whereas the infected animal would survive. There are other factors which very likely also enter into this problem, such as differences in the permeability for arsenic of trypanosomes on the one hand and tissue cells on the other.

"A similar reasoning might also be applied to the unsolved problem of the specificity of the chemotherapeutic action of arsenicals for certain organisms. It is probable that the only infectious diseases that can be benefited by arsenic therapy are those whose causative agents are dependent on a considerable amount of SH compounds for their normal metabolism. If glutathione really plays such an important part in the life of the cell, then it is not at all surprising that substances such as arsenoxid, which react with the SH group of glutathione, should exert a toxic action, and that the latter should be counteracted by supplying the cell with an extra amount of glutathione. In the final analysis, arsenic would have to be considered as a poison which causes death of the cell by interfering with the oxidative processes governed by glutathione."

So stated the suggestion leaves many aspects of the problem unsolved, but it is a step in the right direction in chemotherapeutic investigation and indicates the advisability and necessity for a more thorough study of the chemistry of both body cells and parasites as a means for solving the mystery of the specific tropism of chemical compounds and drugs on the basis of chemical interactions rather than upon the theory of chemoreceptors. Whether or not the specific parasiticidal effects of mercury and bismuth for *Sp. pallida*, or quinin for the malarial organisms, of emetin for endameba, etc., are explainable upon the same basis, remains to be determined; but it is to be hoped that investigations along these fundamental lines will be undertaken in spite of the numerous and great difficuties to be met and overcome in researches of this kind.

Drug Fastness and Tolerance.—The subjects of drug fastness and drug tolerance may now properly engage our attention since the mechanism involved apparently concerns the matters under discussion in an intimate manner. When parasites possess naturally or acquire an increased resistance to the destructive effects of a chemical agent or drug, the state is designated as *drug fastness* or *drug resistance;* when the body cells acquire an increased resistance to the toxic and pharmacologic effects of drugs, the state is desig-

nated as *drug tolerance*. As a general rule, acquired drug fastness of parasites has commanded most attention, but I believe that acquired drug tolerance of body cells involves the same mechanism and is equally worthy of out attention. Both subjects, and especially acquired drug fastness of parasites, have complicated the practical applications of chemotherapy and greatly stimulated Ehrlich in his synthesis of chemical compounds capable of disinfecting the tissues in one dose, *therapie magna sterilisans*, in order to avoid the possibility of parasites acquiring resistance to the compounds.

Acquired drug tolerance has been known for centuries, that is, that the body may gradually acquire increased tolerance for the effects of a drug when given over long periods of time, as, for example, in the case of opium, morphin and cocain addicts, arsenic eaters, etc., but that parasites were capable of undergoing a similar change was not seriously considered as a possibility and practical therapeutic problem until it was discovered that they may acquire resistance to antibodies (serum fastness) and that trypanosomes may acquire fastness to various arsenical and dye compounds.

Natural drug fastness of parasites analogous to the strain of *Tr. equinum* resistant to the arsenicals described by Halberstaedter,[10] is apparently quite uncommon, but acquired fastness is readily produced experimentally and especially with trypanosomes. An extensive literature has accumulated upon this subject as I shall review in succeeding chapters; here it may be stated that not only various trypanosomes, but also various spirochetes and even bacteria, have been rendered drug resistant by exposing the organisms to increasing amounts of the compound either *in vivo* or *in vitro*. Trypanosomes have been rendered resistant not only to such arsenicals like atoxyl, arsacetin, arsenophenylglycin and arsphenamin, but also to fuchsin, azo dyes, trypan-red and trypan-blue. As a general rule, resistance has been conferred by inoculating mice or rats with trypanosomes and treating them with substerilizing doses of the compound; the surviving parasites ("recidives") are then transmitted to fresh animals followed by the administration of a slightly larger but substerilizing dose of drug and so on until it is ultimately found that the organisms can withstand a dose many times larger than required for the complete destruction of the parent strain. Once acquired this fastness persists for very long periods of time and indeed, indefinitely, as shown by hundreds of passages through mice without loss of this property.

Chemical compounds and drugs vary greatly, however, in their ability to render trypanosomes, and presumably other parasites, resistant to their effects. Even among the arsenicals, considerable variation has been noticed. For example, *Tr. equiperdum* in rats is very easily rendered resistant to arsphenamin, while they acquire resistance to arsenious acid very slowly. In my experience, great caution is required in conducting trypanocidal tests with the arsphenamins, because to inoculate a strain in rats previously injected with arsphenamin or neoarsphenamin, may render the strain highly resistant, this accident having occurred in my laboratory several times during the past ten years. As shown by Browning and Gulbransen,[11] even feeding mice infected with *Tr. brucei* with parafuchsin may render the trypanosomes resistant and indicating that the oral administration of drugs in the treatment of disease may have this undesirable effect. Trypanosomes and spirochetes have also been rendered "fast" to antimonials, mercurials and other compounds, as I shall review in later chapters, but experience has shown that fastness is acquired to the dyes and organic arsenicals, especially the trivalent arsenicals, more rapidly and to a higher degree that is true of most other substances.

Specificity of Drug Fastness.—The drug fastness of trypanosomes and other organisms is not absolutely specific. For example, a strain rendered fuchsin-fast is also resistant to many other triphenylmethane dyes, but susceptible to the arsenicals and other dyes, like trypan-red and trypan-blue. Curiously enough, trypanosomes rendered "fast" to arsenic have sometimes shown fastness to antimony, as reported by Morgenroth and Halberstaedter[12] and others. Another interesting exception to the specificity of drug fastness is the fact that dyes of the orthochinoid type, like arsenic-free pyronin, may induce a resistance to the arsenicals, even though they are totally unrelated to the latter compounds; dyes of the parachinoid type like pararosanilin, on the other hand, do not act in this manner. These exceptions to the specificity of drug fastness among trypanosomes and other parasites bear an analogy to similar conditions in acquired tolerance of human beings to drugs. Thus the chronic alcoholic may display an increased resistance to narcotics like chloroform and ether and the morphin addict is said to be sometimes more tolerant to cocain, etc.

But drug fastness in general exhibits a high degree of specificity and a single strain of trypanosomes may be rendered resistant to several different compounds by treating the organisms in succession with them. Ehrlich has even suggested that a strain of this kind may be of service in chemotherapeutic work by providing, as it were, a "therapeutic sieve;" if, for example, a new substance which kills a normal strain of trypanosomes is tested out against a strain possessing a triple fastness, destruction of the latter organisms indicates that the new compound is unrelated to the compounds employed for rendering the strain resistant.

Another curious fact about drug fastness is that a strain of trypanosomes rendered arsenic fast by carefully adjusted passages through mice may not be fast when inoculated into rats; Ehrlich observed this phenomenon quite early in his work and sought to explain it on the basis that in the rat the chemical compound was less organotropic than in the mouse, leaving, consequently, more of it to act upon the trypanosomes which are still susceptible to the compounds. According to this theory, variations in the organotropism or avidity of a chemical agent for body cells would alter the trypanocidal values, and if the body cells of the rat were less susceptible to a certain agent than those of the mouse, it would yield a higher chemotherapeutic index, provided the trypanocidal activities were the same in each animal. As previously stated, the chemotherapeutic index of a compound may vary with different species of animals for reasons not properly understood, unless it is because of variations in the metabolization of compounds in different kinds of animals, which in turn modify both the organotropic and parasitropic effects.

Nature and Mechanism of Drug Fastness.—With this brief review of some of the observed phenomena of drug fastness of parasites and body cells, we may now inquire into the nature and mechanism of these processes. I prefer to discuss these subjects together because it would appear that the mechanism by which body cells acquire an increased tolerance or fastness to a drug is fundamentally the same as the fastness acquired by trypanosomes, spirochetes, bacteria and other protozoan and metazoan parasites, although it would appear that the trypanosomes may acquire fastness to the trivalent arsenicals and some of the dyes much more rapidly than the body cells. It is not necessary, however, to discuss the question of non-absorption of a drug in relation to these processes which may give the appearance of acquired tolerance or fastness, but variations in elimination may properly engage our attention. It is well known, for example, that trypanosomes do

not acquire fastness to the pentavalent arsenicals as rapidly as they do to the trivalent compounds like arsphenamin. Voegtlin and Thompson[13] have shown that this may be largely a matter of rate of elimination as influenced by the physical properties of the arsenicals, the directly toxic trivalent oxid compounds showing the slowest rate, the pentavalent arsenicals the fastest, and the arsenobenzene compounds occupying an intermediate position. In other words, the compound eliminated most rapidly is apt to be least toxic, least parasiticidal and least likely to induce fastness of both body cells and parasites. Furthermore, the apparent tolerance of some animals to a drug due to anatomical and physiological differences need not engage our attention in the present discussion; for example, the absence of vomiting in rodents receiving emetics or the failure of atropin to quicken the heart of the rabbit because its vagus nerves are not tonically active, etc. But tolerance due to increased destruction of a drug like that of morphin by the serum of rabbits, the remarkable natural tolerance of the hedgehog to morphin, atropin, arsenious acid and bacterial toxins, the matter of individual variations in tolerance among animals and the influence of temperature and diet are more pertinent to the question at hand. Ehrlich and his colleagues sought to explain natural tolerance of both body cells and parasites to a congenital absence of suitable receptors for anchoring the chemical compound, drug or toxin; in other words, that such substances may be present in the blood in high concentration, but yet fail to produce physiological or toxic effects because of being unable to enter the protoplasm of cells. Acquired tolerance was explained on the basis of a modification of chemoreceptors by which their avidity for a certain compound and related substances was reduced although not destroyed, since the tolerance may be broken down by increasing the dose or by transferring the resistant strain of trypanosome to some other animal for which the compound was less organotropic, and thereby leaving more of it for action upon the parasites, as, for instance, an atoxyl resistant strain of *Tr. brucei* from the mouse to the rat. However, in this instance it may be that atoxyl in the mouse forms a combination product with some tissue constituent and particularly the liver, as claimed by Levaditi and called "trypanotoxyl," for which the trypanosomes acquire a specific tolerance or fastness, although just as susceptible to a "trypanotoxyl" formed in the rat as would be a normal strain of the trypanosomes. But I believe that instances of natural and acquired fastness of body cells and parasites, not to be explained on the basis of anatomical and physiological variations in the case of the former, will be ultimately explained on a chemical basis, that is, that natural or congenital tolerance is due either to the absence in the protoplasm of a chemical constituent capable of interaction with the compound or drug administered or the presence of a substance capable of union with it followed by rapid transformation to an inert compound. For example, it may be that the real "chemoreceptor" in cells for the arsenicals are the sulphydril compounds mentioned above and that the increased tolerance or fastness of trypanosomes to arsphenamin and its congeners is due to an increase of these above vital needs capable of uniting with and detoxifying the trivalent arsenical oxid believed to be produced when the arsphenamins are administered. At all events, natural and acquired tolerance or fastness to chemical compounds and drugs are not to be explained on the basis of antibody-production and it would appear likely that the theoretical chemo-receptors of Ehrlich will be ultimately shown to be the presence of chemical protoplasmic constituents for which a chemical compound or drug may or may not have the property of union and interaction with a change into non-toxic substances. In the case of arsenic-fast trypanosomes

even physical changes may become apparent; for example, as shown by Gonder,[14] normal trypanosomes (*Tr. lewisi*) can be stained by certain orthochinoid substances and especially triamonphenazselenonium chlorid, causing the blepharoplast to disappear, while arsenic-fast trypanosomes are stained only after their death. At least, the meritorious work of Voegtlin and his colleagues in relation to the SH compounds in body cells and trypanosomes in relation to the toxicity and fastness of the arsenicals opens up this interesting and important field for pharmacologic investigation in therapeutic science. It is true, however, that many other factors must be considered in relation to the mechanism of drug fastness, toxicity and parasiticidal activity of chemical compounds among which may be mentioned the influence of various physical factors, alterations of surface tension, changes in electrical charge, penetrability, etc., and these may now properly engage our interest in relation to the mechanism of drug action upon the body cells and parasites.

Before leaving this subject however, the mechanism of reduction of drug resistance of parasites by passage through some other animal requires brief discussion. For example, if an arsenic-fast strain of *Tr. equiperdum* is passed through several rabbits it is apt to loose entirely or in large part for a time at least its resistance to arsenic when returned to the rat. Voegtlin and his associates believe this change may be due to the chemical composition of the medium followed by a change in the sulphydril system of the parasites. They have also suggested that the resistance of trypanosomes to arsenic induced by subjecting the strain for a long time to sublethal concentrations of such orthochinoid dyes like trypaflavin or pyronin, may be due to the fact that these substances can undergo reduction to leuco compounds and the reaction being reversible, may perhaps affect the sulphydril compounds of protoplasm in the same manner as does arsenic.

At any rate these views are good working hypotheses and it is highly probable that the mechanism involved in the resistance of parasites and body cells to one type of drug is likely to be quite different from that concerned with drugs of an entirely different class. It may be stated however, that drug resistance has nothing to do with virulence and that when a parasite acquires the property it is apparently due to some intrinsic change in its protoplasm which is usually transmitted to succeeding generations for prolonged and indefinite periods of time.

Chemical Action of Drugs upon Body Cells and Parasites in Relation to Toxicity and Parasiticidal Activity.—The key-note of modern chemotherapy is the possibility of synthesizing chemical compounds or discovering natural compounds or drugs of either vegetable or mineral nature, capable of selectively destroying the parasitic causes of disease in the body. The dominant idea is that such effects are brought about by some kind of union with the protoplasm of the parasites. Unquestionably toxic effects may be produced by several factors aside from a direct chemical action upon the protoplasm of body cells by the drug, as, for example, the effects produced by intravascular lysis and agglutination of erythrocytes by arsphenamin, but insofar as parasiticidal effects are concerned, the idea of chemical combination with the parasites (bacterial, protozoan or metazoan) predominates in chemotherapeutic research.

But the evidence of such action is very meager. Does arsphenamin and its congeners cure syphilis by a direct toxic action upon the spirochetes by the compounds as injected, or by trivalent oxids of them produced *in vivo*, or by new compounds of them formed by union with some cellular constituents, like Levaditi's liver "trypanotoxyl?" No one has shown this com-

bination with the parasites by chemical analysis, although the work of Voegtlin and his associates on the interaction of arsenic with the SH compounds of trypanosomes is suggestive. Do mercury and bismuth cure syphilis by a direct union of the compounds (presumably in an ionic state) with the spirochetes? No one has demonstrated such, although there is evidence to indicate that mercury at least tends to accumulate in syphilitic tissues. Does iodin cure syphilis by a direct chemical combination with spirochetes? No one has produced the evidence, although it would appear that this agent may accumulate in gummas and aids in the cure of the disease, not by a direct spirocheticidal activity, but rather by reducing the antitryptic activity of the tissue juices by a saturation of the unsaturated fatty acids and thereby promoting proteolytic activity. Does quinin cure malaria by a direct combination with the malarial protozoa? No one has shown this to be the case by chemical analysis. Are the toxic effects of the organic arsenicals, quinin, emetin, the dyes, mercurials, and chemical compounds and drugs as a whole due entirely or in part to a direct chemical union with the body cells in general or those of a particular organ or tissue? The evidence is not clear, not, however, because of negative results from chemical analysis, but simply because the subject has not been investigated sufficiently to warrant an opinion and especially in relation to the question of chemical action and parasiticidal effects. Indeed, the results of chemical analysis of the liver and other organs after the administration of arsenic, mercury, dyes and other compounds does indicate that chemical compounds have apparently united or entered the protoplasm of body cells, although their exact chemical constitution may not be determined. Organotropic and parasitotropic effects of chemical agents due to definite chemical combination with constituents of the protoplasm have not been disproved, and while the acceptance of such a theory is difficult because the same toxic action may be produced by drugs having no chemical reactions in common, yet this should not weigh too heavily against the view that the toxic and parasiticidal effects of some chemical agents and drugs may be due to a definite chemical combination with the affected cells. At least the tendency for some drugs to accumulate in certain cells, the relation between biological action and chemical constitution, the fact that many compounds are altered in the body, as, for example, the arsenicals by processes of oxidation and reduction, tend to confirm this view, although it is freely admitted that these effects may occur as a result of physical properties and that instead of definite chemical combinations merely adsorption occurs; in any event, the combinations may be very very loose and unstable and the effects produced only as the agent passes in or out of the cells.

The subject is one of fundamental importance in relation to pharmacology, toxicology and therapeutic science, long and persistently neglected by the investigator because of the many, great and, at the present time, apparently insuperable difficulties attending investigations along these lines. But the basic idea of pharmacologic action, toxicity and parasiticidal effects being due in part, at least, to definite chemical combination of drugs with protoplasmic constituents has not been disproved by any means, and I believe it is worthy of very serious consideration as a fundamental mechanism. I have already discussed the subject of chemical constitution of compounds in relation to drug fastness, organotropism and parasitotropism, pointing out that changes in chemical constitution may profoundly alter these actions, but this may be not only a result of changes in the physical properties and physical action of the compounds, but also because of an alteration in the chemical affinity of the compounds for some constituents the body cells and parasites.

Physical Reaction and Effects of Drugs upon Body Cells and Parasites in Relation to Toxicity and Parasiticidal Activity.—But while the definite chemical action of drugs upon body cells and parasites bears this important relation to pharmacological, toxicological and therapeutic activities, within recent years there has been a growing tendency to ascribe these actions in whole or in part to various physical properties and action. However, these in turn are usually influenced by the chemical constitution of the drug, thereby preserving in an indirect way the importance of chemical constitution in relation to biological action, but the effects of the drug are ascribed primarily to such physical characteristics as solubility in the blood and tissue juices, solubility in the cells, adsorption by the cells, changes in electrical charge, salt action or penetration of cells with a drug and a consequent alteration of protoplasmic constituents in relation to the surrounding fluids, alterations in surface tension of cells, changes in the cell membranes of body cells and parasites by fat-solvents, protein precipitants, etc.

Probably one of the most important of these physical factors is their influence upon the *permeability and penetrability of cells*. Thus Traube and Matthews state that the capacity of adsorption by the cell substance is of the greatest importance and that every transition causes far-reaching changes. For this reason, even sodium chlorid may become a protoplasmic poison in certain concentrations strongly influencing the colloidal state of the proteins of protoplasm. Adsorption changes consist principally of a sedimentation of colloids with an interaction between the electrical charge of the smallest particles of the cells and precipitating substance, and "as the products of microörganisms probably always will be cationic and anionic, the most favorable effect will be gained by combination of drugs composed of cationic and anionic preparations." As shown by Moldenhauer,[15] in experiments upon the penetration of pentavalent and trivalent arsenic into the wall, protoplasm and sap of *Valonia*, the highest concentration was found in the protoplasm, pointing to a combination of arsenic with protoplasm, but the degree of penetration was least when the external solution was nearly neutral and increased as the H ion concentration became either more acid or alkaline. For example, trivalent arsenic did not penetrate as readily into protoplasm in the acid range as pentavalent arsenic, showing the influence of reaction of the surrounding medium upon the process.

The penetration of dyes and arsenic into the brain and other tissues is likewise apparently influenced by solubility reactions. Ehrlich,[16] for example, stated that neurotropic substances are lipotropic, and Overton[17] believed that before a substance could penetrate into a cell it must be soluble in the cell membrane, and since these membranes were regarded as being lipoidal it should follow that a drug to penetrate must be soluble in lipoid solvents. That this subject is of therapeutic importance is indicated by the experiments of McIntosh and Fildes[18] upon the penetration of dyes and arsphenamin for the brain; these authors were inclined to believe that the absence of arsphenamin in the brain was due to the fact that it was not soluble in chloroform or related substances. Likewise with the dyes the chief factor governing their passage from the blood into the tissues appeared to be a peculiar solubility reaction, but not a question of general lipoid solubility. Langer,[19] for example, believes that the efficacy of a bactericidal agent depends on its power to penetrate the exterior of bacteria and that this in turn is closely associated with its diffusibility and on the electrolytic qualities of the semi-colloidal solution.

The whole subject of solubility and penetration is, therefore, of great interest in relation to chemotherapy and the recent lectures of Hamburger[20]

upon the significance of permeability—problems for the biological and medical sciences, are examples of the increasing interest and importance of these physical or physico-chemical factors in pharmacology and therapeutic science. And permeability and penetration are not only of primary importance in relation to organs and especially foci of infection like streptococcus and staphylococcus abscesses, pneumonic lungs, tubercles, syphilitic deposits, etc., but likewise, and even more importantly, in relation to the penetration of individual cells and especially of the various pathogenic bacteria, molds and fungi, protozoa and metazoa. Indeed, these questions are of fundamental importance in chemotherapy and urgently require systematic investigation.

However, since the influence of physical factors in relation to pharmacological action of drugs are generally well known, they need not be discussed here in detail, although of primary importance in relation to the toxicity of chemical compounds for both body cells and parasites. For example, a large number of chemotherapeutic compounds are administered by intravenous injection and at once the problem of toxic reactions due to physical changes in the blood becomes important. As I shall discuss with more detail in later chapters, it is probable that immediate reactions following the intravenous injection of arsphenamin, mercury and other compounds may be due to intravascular lysis, agglutination or precipitation of plasma proteins and salts. But changes in the cellular membranes of both body cells and parasites with acceleration or retardation of permeability and alteration in electric charges may influence the passage of free ions and either increase or decrease toxicity for both body cells and parasites. These surface forces are in turn greatly influenced by changes in hydrogen-ion concentration, lipoid solvents, salts, etc., with possible changes in the aggregation of the protoplasmic colloids. For example, the action of phenol on bacteria is largely dependent upon its being more soluble in lipoids and in proteins than in water and upon its consequent concentration in organisms. Its parasiticidal activities are decreased, therefore, by any condition which decreases its differential solubility like alcohol, alkali, and oil. The action of the cresols and coal-tar acids appears to depend upon emulsions being absorbed by organisms and the action of mercury and other heavy metals is apparently due to absorption upon the surface followed by penetration into the interior of cells. The effects of electrolytes and proteins in diminishing the oligodynamic or parasiticidal activity of chemical agents are likewise examples of the influence of physical forces as, possibly likewise the effects of chlorin compounds and oxidizing agents due to the union of free chlorin or oxygen with the proteins of body cells and parasites. The effects of mercury, silver and other heavy metals are generally ascribed to ionic dissociation, as originally shown by Krönig and Paul with mercuric chlorid, but differences in degree of ionization will not explain all differences in the disinfectant activity of such compounds; for example, mercuric nitrate and acetate dissociate as freely as mercuric chlorid, but yet these do not possess by any means the bactericidal activity of the latter.

But while pharmacologists have discussed such questions at length in relation to the pharmacological and toxicological actions of drugs, they have not always attempted to explain parasiticidal effects on the same basis. It is reasonable to assume, however, that parasites are subject to the same changes and just as the selective effects of drugs for certain cells are explained on the basis that they are peculiarly susceptible to this or that physical effect and change, so may we assume that it is possible for selective parasiticidal effects to be influenced by similar physical actions. But in the

final analysis it would appear that these physical phenomena are primarily influenced by the chemical state of the protoplasm of body cells and parasites, the surrounding fluids and by the chemical constitution of the drug. As so well stated by Cushny: "These views have all been supported by a certain amount of evidence, and there is every reason to believe that these physical properties are important factors in the action of some drugs. But it is equally obvious that no one of them will explain the whole of pharmacological action, and there is reasonable doubt whether the whole of the physical characters taken together will suffice for this. From the present confusion the only legitimate conclusion seems to be that the activity of drugs depends on a large variety of factors, and that pharmacological action cannot be brought under any one law, either chemical or physical."

McDonagh,[21] however, has attempted to place chemotherapy upon definite physico-chemical laws of oxidation and reduction; for example, in his opinion, the arsenic of arsphenamin does not attack *Sp. pallida* directly, but only indirectly by increasing the oxidizing action of the protein particles in the plasma and cells. McDonagh believes that in chemotherapy metals cause dispersion of the protein particles and non-metals condensation, the former conducting electricity to the particles causing them to increase in number and increasing their negative charge; that the chief protective substance is the protein in the serum, which exists in the form of colloidal particles, each of which has a surface; that the parasitic cell is nothing more nor less than a number of colloidal particles enclosed in a fixed area; that all actions between colloids are surface actions, and the two most important are those of oxidation and reduction; that parasites require oxygen for their existence, and, always living at a disadvantage, must needs protect their oxygen, which they do by exhibiting a prevailing reducing action on their surface; that the colloidal particles of the protective substance (antibody) strive to increase their surface oxygen, which appears to exist in the form of hydroxyl (OH); that as hydroxyl and hydrogen possess opposite electric charges, they will neutralize each other when they meet; that, if, then, the protective substance has an excess of hydroxyl it will destroy the hydrogen on the surface of the parasite, with the result that the latter will die because it will be unable to reconvert the metallic tri-hydroxid into the di-hydroxid or catalytic form; that, therefore, the aim of chemotherapy is to increase the hydroxyl on the surface of the protective substance.

I am stating McDonagh's views much as he has stated them, but my training and experience do not permit me to discuss them, compelling me to merely give the chief principles and some of the references for the aid of those particularly interested in these views, which, at least, have the merit of originality.

Distribution, Storage and Fate of Chemical Agents within the Body in Relation to Chemotherapy.—In chemotherapy, as in therapeutics in general, chemical compounds are not distributed uniformly, but accumulate especially in certain cells, according to chemical affinities or physical forces of which permeability, absorption and adsorption would appear to be of great importance; probably in most, if not all instances, distribution after absorption is the result of a combination of chemical and physical forces, and the aim of chemotherapy is to produce chemical agents possessing a selective affinity for invading parasites with a minimum of affinity for body cells and especially cells of physiological importance whose injury or death would produce lesions and symptoms and prevent the use of the compound regardless of what parasiticidal activity it may possess. In other words, the distribution of a compound in the tissues following absorption after enteral or parenteral

administration is intimately related to its organotropic and parasitropic properties, although both of these in turn are subject to modification by disease states.

But as a general rule, chemical compounds undergo more or less profound changes after absorption and with few exceptions are not eliminated in exactly the same chemical state as when administered. With the metallic compounds like those of mercury, silver and gold, this may amount only to a varying degree of ionic dissociation; but with the dyes and arsenicals, changes of oxidation or reduction may occur profoundly influencing distribution, organotropism and parasitotropism. It is largely for this reason that the results of *in vitro* parasiticidal tests have but little bearing upon possible *in vivo* effects, although a more direct relation exists in regards to the effects of the agent when applied locally to the skin or a mucous membrane under which circumstances there are far less chances for the profound changes occurring after absorption into the blood and other body fluids. Insofar as parasiticidal effects are concerned, the changes *in vivo* may result in an increase of activity as apparently occurs with the organic arsenicals undergoing oxidation or reduction into the trivalent oxids; or a reduction in activity as occurs with practically all the dyes, acriflavin being a possible exception. As a general rule, however, whatever increases toxicity for the parasites also tends to increase toxicity for the body cells, although the ratio may be so much in favor of the former as to endow the compound with therapeutic properties.

As a general rule, the most profound changes occur after absorption. In the gastro-intestinal tract, hydrolysis, saponification and various other changes due to either the acids or alkalies of the stomach and intestines may materially alter a compound, but the important changes of ionic dissociation, oxidation and reduction occur mostly after absorption.

Naturally, the tendency of these forces is always to reduce toxicity for the body cells. The primary change may increase toxicity for both body cells and parasites for a brief time, but the ultimate result is a reduction. For example, the pentavalent and trivalent arsenicals, reduced or oxidized to the toxic trivalent oxids, appear to be finally transformed into more or less inert pentavalent compounds; mercury and many metals are detoxified by union with proteins; phenols and other aromatic compounds by sulphates, etc., the degree and extent of such activities depending upon the activity of metabolic processes and the amounts of neutralizing substances available. The liver and muscles are particularly concerned in disintoxication processes, especially the former for the arsenicals, quinin, the metals, etc., although no symptoms may arise from this organ. It is likely that the liver is involved in this manner largely because of its eliminative functions, just as the kidneys, skin and gastro-intestinal mucosa may be found to contain unusually large amounts of various chemical agents, not because of selective affinities, but because they are brought to them for elimination, during which process, however, and by reason of such function, they may suffer injury, as shown by the effects of mercury upon the gums, lower gastro-intestinal tract and kidneys.

Naturally, these forces influence the concentration of chemical compounds and drugs and our methods of administration. When injected intravenously or absorbed from the muscles or gastro-intestinal tract, they rapidly disappear from the blood and the concentration in the tissues depends primarily upon the dose given, rate of absorption, rate of excretion, rate of change into harmless substances, etc., and the problem in chemotherapy is to raise or maintain a concentration of parasiticidal proportions without injury to the

body. If accumulation occurs in any organ or organs exceeding the rate of elimination, toxic cumulative effects may occur as sometimes met with in the administration of mercury, arsenic, the iodids, etc.; or these and other compounds employed in chemotherapy may cause continually repeated injuries, individually too slight to be noticed, but ultimately producing symptoms as a result of such damage, rather than by accumulation of the compound.

This brings us to a consideration of the question of the therapeutic activity of stored or accumulated compounds so frequently referred to in chemotherapeutic literature as "depots." Does the arsenic stored in the liver during the treatment of syphilis with arsphenamin and its congeners exert spirocheticidal or other therapeutic effects in syphilis? Unquestionably it may injure the liver in the way of producing albuminous and fatty degeneration, but I very much doubt that it exerts a therapeutic effect, as will be discussed more fully in Chapter XII. Does mercury accumulated in a tissue or organ, probably as a loose globulin compound, continue to exert spirocheticidal properties? In my opinion this is highly probable, since it would appear that absorption and dissociation may not be involved. The matter depends therefore, upon the state or condition of the stored chemical agent insofar as therapeutic activity is concerned. Merely because a compound like arsenic is stored, need not necessarily mean that it will exert a curative activity in syphilis when re-absorbed and in the process of elimination. But we have very little information on this subject, although I think the little we have warrants the statement that "depot therapy" may or may not be of value in chemotherapy, according to the compound administered: compounds stored in a therapeutically inert state like some pentavalent arsenicals are probably inert while compounds of the metals like mercury and bismuth, may be active if capable of dissociation upon re-absorption.

Influence of Disease upon the Toxicity and Parasiticidal Activity of Chemical Compounds in Relation to Chemotherapy.—As is well known, various pathological states may profoundly alter the absorption, distribution, elimination and general effects of a chemical compound. This subject is of special importance in chemotherapy, for while the pharmacologist and therapeutist may administer a drug for its effects on healthy tissues in order to relieve the diseased tissue of some or all of its work and to permit it to rest, the chemotherapeutist aims to disinfect the diseased tissue. For example, in valvular disease of the heart, drugs may be given, not with the hope of restoring them to health, but to act upon the healthy heart muscle to correct disturbances of circulation due to the defective valves. But chemotherapy primarily aims to cure the diseased tissue, although it is sometimes resorted to for its general effects, as the administration of neoarsphenamin in the treatment of some of the non-syphilitic anemias.

There is, however, a very peculiar relation between some diseases and the pharmacological and toxicological effects of chemical compounds. For example, the bromids may suppress the convulsions of epilepsy with much less suppression of the brain of normal individuals; digitalis may produce diuresis in cardiac disease but not in health; antipyretics may reduce the temperature in hyperpyrexia but not when the temperature is normal; quinin may produce cinchonism of normal persons in small doses, while malarial and pneumonic individuals ordinarily withstand large doses; the iodids may rapidly produce iodism in healthy persons, while syphilitics can sometimes take enormous doses; tolerance for mercury may be slight in the healthy, while greatly increased in syphilis, and so on through quite a list of examples. In other words, it would appear that the toxic effects of some compounds like the iodids, mercurials and even the arsphenamins, are appreciably reduced by the presence of *Sp. pallida* in the body and that the toxic effects of quinin and related substances are reduced by the presence of the malarial protozoa and pneumococcus just as the virus of acute rheumatic fever appears to greatly increase tolerance for the

salicyl compounds. Why this is so cannot be stated; probably it is due in part to more rapid metabolization of the compounds like the effects of alcohol in hyperpyrexia, or to some peculiar detoxifying influence by products of the parasites. On the other hand, just the very opposite effects may occur with an appreciable reduction in resistance or tolerance to drugs, and especially in acute infections, by reason of a probable reduction in what is commonly designated as "general resistance." For example, white rats heavily infected with virulent trypanosomes cannot ordinarily withstand as much arsphenamin per kilogram of weight as normal rats; in other words, there is a summation of the toxic effects of the infectious products and the drug along with a suppression of vital resistance. Of course, the influence of diarrhea upon reducing absorption when drugs are administered orally by rushing them through the canal to the external world is readily understood; likewise the very opposite to this, when accelerated absorption may occur by more rapid diffusion in diseased intestines; also the increased absorption of some compounds after hemorrhage and especially increased absorption by the meninges and brain following dehydration of these tissues.

When the enunctory organs are involved by disease, there is always an increased danger from toxic effects. For example, the intravenous injection of ordinarily harmless doses of mercury or arsphenamin in the presence of nephritis may result in severe nephritis and anuria; arsphenamin administered to individuals with a damaged liver may greatly increase the chances of arsenical jaundice, just as mercury or bismuth greatly increase the chances of producing siderosis and gingivitis in the presence of pyorrhœa alveolaris.

In other words, the presence of pathological states may profoundly influence the absorption, distribution and elimination of chemical compounds and these in turn their organotropic or toxic effects. In chemotherapy especially these influences must be kept in mind and particularly when physiologically important organs are involved. In a general way, it may be stated that the presence of parasites in the tissues influences the localization of some chemotherapeutic compounds probably by reason of a peculiar chemical or physico-chemical affinity between some protoplasmic constituent and the drug; for example, I have reasons for believing that mercury may especially accumulate in syphilitic tissues even when bone is not involved and certain dyes and other agents are known to collect in tubercles.

This naturally brings up the question of what may be the true value of animal experimentation in pharmacological and chemotherapeutic investigation when normal animals are employed and especially in relation to the toxicity of chemical compounds and drugs, if these effects are so likely to vary among human beings and the lower animals and so subject to modification by diseased states. As stated by Sollmann, "this brings up the fundamental question: To what extent can results observed on animals be transferred to man? The same question applies to all other fields of experimental medical research, and has been abundantly answered by their results" and I shall reserve for the succeeding chapter a more detailed discussion of the subject in relation of chemotherapy.

Influence of Chemical Compounds upon Immunological and Tissue Reactions in Relation to Chemotherapy.—But while the pharmacological and toxicological action of chemical compounds and drugs may be influenced by pathological states, either in the direction of reducing or increasing resistance or tolerance, the influence of drugs upon tissue reactions about areas of infection may have a beneficial influence with an increase of parasiticidal activities. I refer to the possibility of increasing hyperemia, leukocytic infiltrations and the laying down of fibrous tissue, known as the *dynamogenic activity* of chemical compounds and believed to be of beneficial influence in such diseases as tuberculosis and syphilis by reason of circumscribing the infection, shutting off oxygen and food supplies and allowing the acid and other waste products of the organisms to accumulate and gradually

bring about their destruction. It is sometimes stated that arsenic may exert beneficial effects of this kind; that gold compounds may produce hyperemia about tubercles resembling the tuberculin reaction and that various substances like cinnamic acid, phosphorus, nucleinic acid and silicic acid may excite inflammation and promote mineralization of the tissues of tubercles.

Of equal importance is the possibility of some compounds uniting with cellular constituents and forming actively parasiticidal compounds. Levaditi and Yamanouchi, for example, believe that arsphenamin and other therapeutically active arsenicals owe their efficacy to the production of active compounds with liver cells and the whole subject of an important synergistic activity on the part of tissue constituents, with the production of new compounds of enhanced parasiticidal properties, is worthy of consideration, although the direct evidence in view of this hypothesis is meager at best and largely based upon the assumption that such combination products must be produced *in vivo* to which therapeutic activities may be ascribed.

But the influence of chemical compounds upon chemotaxis, phagocytosis and other immunological processes has long commanded most attention in relation to chemotherapy. These effects may be due, however, to two entirely different mechanisms, namely, (*a*) the possibility of chemical agents destroying parasites and thereby liberating antigenic substances capable of engendering antibody-production and (*b*), the possibility of the agents directly stimulating the antibody-producing tissues. Ehrlich always maintained that the former process was of importance in chemotherapy and that chemical agents may release antigenic substances from parasites with increased antibody-production, or so far devitalize organisms and especially their proliferative activities, that natural or acquired immunological principles were enabled to finish the job of destruction. Ehrlich [22] also thought that these effects may bring about paradoxical results if large numbers of parasites were suddenly destroyed with the release or production of large amounts of toxic substances producing what he termed "*effectus contrarius.*"

Needless to state, any chemical compound producing leukocytosis may increase the parasiticidal activities of the blood and especially if phagocytosis were promoted. Probably the beneficial effects of mercurochrome by intravenous injection in staphylococcus and streptococcus septicemias may be due in part to the induced leukocytosis and the release of proteolytic and lipolytic ferments from these cells. In the chemotherapy of localized infections, the influence of chemical compounds upon phagocytosis is of particular importance, as it is so easy for drugs to paralyze the phagocytic activities of microphages, macrophages and clasmastocytes instead of promoting phagocytosis. As shown by Felton and Dougherty,[23] with four cinchona derivatives injected intraperitoneally in mice, a chemical agent may be chemotactic and increase leukocytic exudation, but yet prove antiphagocytic and therefore actually harmful. In my experience, however, drugs capable of paralyzing leukocytes are generally somewhat stimulating in higher dilutions.

It is also important to bear in mind the possibility of chemical agents reducing parasiticidal effects by a destructive action on complement and various lytic types of antibodies in the blood and especially in tissues being treated by local applications. The whole subject is one of commanding interest and in the following chapters I shall refer to the influence of various chemotherapeutic agents upon immunological processes in a discussion of the mechanism of their parasiticidal activities.

But can it be said that chemical agents are capable of stimulating antibody-production by a direct action upon the antibody-producing tissues?

It is commonly stated that arsphenamin and mercury cure syphilis by stimulating antibody-production. I believe it is possible that these drugs owe part of their curative activity in syphilis to bringing about the destruction of *Sp. pallida* with the release of antigenic substances from them, but I have had no direct or convincing evidence at all that any chemical compound or drug is capable of actually increasing antibody-production by a direct stimulation of the antibody producing tissues, although they may increase the numbers of leucocytes in the blood and around disease foci with a possible release of enzymes capable of producing therapeutic effects, as Bergel[24] has suggested in tuberculosis in reference to the release of lipolytic enzymes from lymphocytes, which might be imagined to increase the vulnerability of tubercle bacilli to destruction by hostile drugs or other substances.

Combined Action of Drugs in Relation to Chemotherapy.—Related in a general manner to these subjects of the mechanism of toxic and parasiticidal activities of chemical compounds and drugs and especially of drug fastness, is this subject of the combined action of drugs. It is, indeed, an old and much discussed subject in therapeutics but has been revived within recent years in relation to chemotherapy. As stated by Ehrlich, the principle of combination chemotherapy, "is to attack the enemy simultaneously from two or three sides, and thus to produce a completely successful result by the combination of several different substances, each of which alone does not show a sufficient effect. Thus it has been found by Laveran and by Franke that certain infections which are influenced neither by trypan-red nor by atoxyl alone, can be cured if these two substances are used in conjunction. I, too, have in course of time repeatedly met with similar cases, and am convinced that substances which by themselves are not very efficient, may yet produce good results if they are used as adjuncts to those more powerful, though not completely curative, substances. Supposing, for example, a highly trypanocidal remedy to kill ninety-eight among one hundred parasites; its administration would be followed by an instantaneous improvement, but not a definite cure, since the two surviving trypanosomes must in course of time produce a relapse. But if one combined this powerful remedy with another which, even though much weaker, yet sufficed to kill just those two surviving but attenuated parasites, then such a combination would serve to bring about the otherwise unattainable cure."

At another time, Ehrlich stated that the principle of combination chemotherapy is to "march separately, strike combined," and that in this connection "it must be observed that for the combination therapy such drugs must be chosen which will find in the parasite various chemoceptors. For instance, it would be purposeless to employ two arsenicals with bi- or tri-valent arsenic; also fuchsin should not be combined with the allied methyl-violet, as the same receptors are present, but the fuchsin bodies should be combined with arsenicals. Two different remedies may eventually be introduced in very small quantities into the organism without harming it and also without losing their parasiticidal qualities. On the contrary the relation between the curing dose, *dosis curativa*, and the toxic dose, *dosis toxica*, becomes much more favorable and far smaller than when the single remedies enter into action each by itself. By such a combination of different classes or types of dyes and arsenicals which have also a different distribution, the principle of marching 'separately and striking combinedly' is put into effect. Especially such drugs should be chosen which attack the two cell constituents, nucleus and protoplasm, separately."

Furthermore, it would appear that under combination therapy, drug fastness may be overcome in practical therapeutics and may be even made to

disappear if one may accept reports indicating that patients with malaria treated with quinin and rendered quinin-fast were later found sensitive to quinin after treatment with arsphenamin. In syphilis it is possible that the spirochetes may acquire fastness to the arsenicals but remain sensitive to the mercurials or bismuth and the reverse.

Another example of combination therapy is the treatment of bacterial infections with immune serum and chemotherapy, known as chemoserotherapy.

Numerous additional examples of combination chemotherapy may be cited and especially in the treatment of trypanosomiasis with the arsenicals and antimonyls; the treatment of syphilis with the arsenicals, mercurials and bismuth salts; the treatment of malaria with the cinchonics, arsenicals and methylene blue, etc., but I shall return to the subject in later chapters upon the chemotherapy of these and other diseases.

Naturally Ehrlich considered combination chemotherapy a complete support for his theory of chemo-receptors, but this is not necessarily so since it would appear easily possible for chemical agents of similiar parasiticidal properties to attack along the chemical or physico-chemical lines discussed above. Neither is it a justification for the use of indiscriminate and "shot-gun" mixtures in therapeutics, since in chemotherapy combination therapy is strictly limited to the combined use of the fewest possible drugs known to possess some degree of specific curative effect for the disease under treatment. As a general rule, the compounds are administered separately but may be mixed together before injection, as in mixtures of neoarsphenamin and mercuric chlorid, with the possibility of forming new compounds either before or after administration with synergistic effects. On the other hand, due care must be exercised against the adoption of incompatibles and antagonistic combinations. As a general rule, it would appear that combination therapy in chemotherapy is usually a simple algebraic summation of the effects, but not infrequently the results cannot be explained on this basis and a potentiated effect is observed. The mechanism of potentiation has not yet been elucidated although Buergi's law[25] would appear applicable to some of the phenomena, namely, that potentiation occurs always and only, when two compounds have different points of attack in the cell because two different receptors take up more poison in a given time than could a single receptor from a double quantity of a single compound. Naturally, Ehrlich was inclined to accept this theory since it called into play his own theory of chemo-receptors, but I believe the subject may be reduced to a simple chemical or physico-chemical proposition in which two or more drugs may find in the same parasite protoplasmic constituents for which they have a chemical affinity and with which they may interact independently of each other; or two or more compounds may interact before or after reaching cells with the production of new compound capable of chemical or physico-chemical interaction with protoplasmic constituents. From the standpoint of chemotherapy, combined therapy to be useful must have more parasiticidal effects than either drug alone without, however, an increase of organotropic effects.

Summary of the Nature and Mechanism of the Toxic Effects of Chemical Agents and Drugs for Parasites and Body Cells.—It is apparent, therefore, that chemotherapy has developed into a far more complex subject than originally conceived by Ehrlich, with a need for modifying the theoretical structure upon which it was based. The ideal and aim of producing chemical agents capable of selectively disinfecting the tissues without harm to the host remains unchanged, but I have all but abandoned the hope of accom-

plishing this on the basis of the chemo-receptor theory. Complex chemical and physico-chemical forces are involved in the effects of a chemical agent on both body cells and invading parasites and the matter of selective destruction of parasites in the tissues would appear to be one of chemical or physico-chemical interaction between the chemical agent and protoplasmic constituents of the parasites, just as the specific immunological processes of the body are being gradually placed on the same basis.

Instead of conceiving a remedy killing invading organisms by direct union through the agency of theoretical chemo-receptors, a broader and more comprehensive conception is required to explain the observed phenomena, as a remedy not acting as such, but in virtue of the formation from it in the body of some directly toxic product, either by a modification of its structure or by its union with some tissue constituent; of modifications of virulence and a reduction of resistance with increased susceptibility to destruction by the body's natural defenses, the *mechanism of parasiticidal activity* calling into play any one or several of the following factors depending upon circumstances and discussed above in detail:

1. A direct chemical interaction between the compound or drug as administered or after some transformation in the body, with some protoplasmic constituent of the parasite, resulting in the death or crippling of the latter by interference with its vital processes as those of alternate oxidation and reduction (internal respiration).
2. A physical or physico-chemical interaction with the protoplasmic colloids of parasites involving precipitation, coagulation, changes in electrical charge, etc., sufficient for destruction or crippling.
3. The possible production of new compounds in the tissues capable of chemical or physico-chemical interaction with protoplasmic constituents of invading parasites rather than the production of these effects by the compound direct as administered.
4. The possible production of antibodies by releasing antigenic substances from the parasites.
5. The stimulation of processes of oxidation; the production of hyperemia and stimulation of reparative processes; the production of leukocytosis and mobilization of proteolytic, lipolytic and diastatic enzymes capable of interfering with the nutrition or otherwise crippling invading organisms, etc.

And the *mechanism of organotropic or toxic effects* of chemical agents for the body cells in general or of a particular group, is likewise far from being the simple matter of union by chemo-receptors, involving as it does the subjects of absorption, elimination, distribution, fate and other complex pharmacological and toxicological effects, the production of toxic lesions and symptoms being by one or more of the following processes:

1. By immediate reactions in the blood like agglutination or lysis of erythrocytes, precipitation of plasma proteins or salts, with the production of colloidal shock reactions, embolism, etc.
2. By direct chemical interaction between the compound or drug as administered or after some transformation in the body, with some protoplasmic constituent of the fixed body cells and resulting in the death or crippling of the latter by interference with vital processes. Or by some physical or physico-chemical interaction with the protoplasmic colloids of cells involving precipitation, coagulation, changes in electrical charge, etc., either mechanism involving the body cells as a whole or particular groups and centers by reason of special affinities or through the function of elimination.
3. By producing excessive hyperemia, cellular infiltrations, and other tissue changes; by promoting excessive enzymic activity with the production of toxic substances from dead or devitalized body cells or parasites; by promoting or stimulating the activities of parasites, as in the Jarisch-Herxheimer reactions of syphilis, etc.

A study of the toxicity of a chemical compound or drug, therefore, in relation to chemotherapy, may be not only a matter of simply determining the minimal lethal or maximal tolerated doses per kilogram of body weight for the lower animals, but under certain circumstances involves a study of

its pharmacological and toxicological effects for human beings and especially for sick human beings, which brings into play far more complex processes than met with in healthy lower animals; but this is a large and important subject which may be taken up in the succeeding chapter.

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CHAPTER II

METHODS FOR DETERMINING THE TOXICITY OF CHEMICAL AGENTS IN RELATION TO CHEMOTHERAPY

Unfortunately, the biological properties of a compound or drug, that is, its pharmacological, toxicological and parasiticidal activities in the body, cannot usually be determined by chemical analysis alone; as a general rule, these are ascertained empirically or by tests employing the lower animals. It is true, however, that chemical analyses are frequently helpful in these respects; for example, to know beforehand the amount of arsenic present in an arsenical compound or the amount of mercury present in a mercurial compound, etc., will sometimes enable one to estimate the probable toxic doses for the lower animals, but such information is seldom acceptable in lieu of actual toxicity tests. This is particularly true of complicated organic compounds; no matter how carefully they are prepared and that analyses indicate that several of a kind are apparently identical in chemical constitution, rigid animal tests sometimes show existing variations in toxicity and to a lesser extent of parasiticidal activity.

Empiricism in Relation to Chemotherapy.—But before entering upon a discussion of the value and limitations of animal experimentation in relation to chemotherapy, it may be well to briefly refer to the rôle of empiricism in

the same relation. Merely to mention that empiricism alone gave us quinin for malaria, mercury for syphilis, the salicyls for acute rheumatic fever, ipecac and emetin for amebiasis, tartar emetic for kala-azar and other forms of leishmaniasis, chaulmoogra oil for leprosy, etc., is sufficient for indicating the very important rôle it has played in the development of chemotherapy.

It is entirely likely as stated by Dale[1] that empiricism will continue to be an integral part of chemotherapeutic advance and under certain conditions is indispensable. When new compounds are synthesized, they should not be administered to human beings until some adequate idea of their toxicity has been gained by administration to the lower animals and especially if intravenous injections are to be given. To rush a new compound into clinical use before its toxicity at least has been estimated by such preliminary tests is frequently dangerous and always unnecessary and reprehensible. But after this has been done and a compound has been used clinically for the treatment of a given infection, there is no harm in trying it in the treatment of another disease and especially if it is caused by a biologically related parasite. Furthermore, some diseases of human beings cannot be reproduced or duplicated in the lower animals and preliminary parasiticidal tests, therefore, are sometimes impossible; but this does not contra-indicate the use of the compound clinically, provided always that we know in reasonably accurate terms the toxicity of the compound so that one may proceed on the basis of possibly securing a good therapeutic result while doing no harm. In other words, we are never justified in administering a new and unknown compound to human subjects for the treatment of disease, until its capacity for harm has been first determined with reasonable accuracy; on the contrary, however, with such information at hand, we are frequently justified in using it empirically before its possible therapeutic effects have been determined, as this may be only possible by actual administration if the infection cannot be reproduced satisfactorily in the lower animals. Furthermore, there are some rather sharp limitations to the value of pharmacological, toxicological and parasiticidal tests employing the lower animals which may be now discussed with more detail, but the fact requires emphasis that valuable information on the matter of toxicity is always to be gained by properly conducted tests among the lower animals and that no compound potentially harmful should ever be administered to human beings until such tests have been conducted.

Value and Limitations of Experimental Pharmacology in Relation to Chemotherapy.—Usually it is only after a new compound has been quite thoroughly studied from the standpoint of toxicity and therapeutic activity in the lower animals and has found a place of usefulness in the treatment of disease, that its pharmacology attracts attention. But knowledge bearing upon its absorption, fate and excretion, as well as its effects, if any, upon the higher and important centers, heart, etc., becomes indispensable for rational therapeutics; for determining these and related questions, experimental pharmacology may render valuable services.

But great care must be exercised in applying the results of experimental pharmacology to man. Differences in the physiological functions of the lower animals, natural resistance and individual idiosyncrasies, seasonal variations, temperature and diet may have a marked effect on the resistance of the lower animals, as I shall discuss shortly with more detail in relation to toxicity tests; but, as stated by Sollmann,[2] in the hands of the expert pharmacologist, errors of interpretation of results due to these and related subjects are not likely to occur.

As stated in the preceding chapter, pharmacology and chemotherapy are inseparately united, the former being in a way the parent of the latter. I shall, therefore, in later chapters aim to present the pharmacology of the more important chemical compounds and drugs employed in chemotherapy as this knowledge is of the greatest value in therapeutic science; and not a small part of this knowledge has been gained by animal experimentation, although in this treatise the technic of pharmacological methods need not be described, as these are highly specialized and are to be found in text-books devoted to experimental pharmacology.

Value and Limitations of Parasiticidal Tests with the Lower Animals in Relation to Chemotherapy.—While chemotherapy has been applied empirically in the treatment of some metazoan diseases, yet, from the standpoint of the treatment of experimentally produced infections in the lower animals, most investigation has been devoted to protozoan and bacterial diseases and especially those produced by various pathogenic trypanosomes and spirochetes.

Indeed, the discovery of methods of producing experimental trypanosomiasis in mice and rats by Mesnil and Brimont gave great impetus to the original investigations of Ehrlich, Bertheim, Hata and their associates; this was soon followed by the adoption of experimentally produced spirochaetic infections, notably spirillosis of chickens, relapsing fever of mice and syphilis of rabbits, all of which have proved of the utmost value in chemotherapeutic research and contributing in a very essential and important manner to chemotherapeutic discoveries in dye and arsenical compounds. During the past ten years, additional methods have been worked out for the production of other protozoal diseases, as well as of numerous bacterial diseases, so that experimental infections of the lower animals have proved indispensable and capable of yielding information of paramount importance in chemotherapeutic investigations and the practical treatment of disease of human beings. Indeed, it may be stated, that without animal experimentation many of the present great discoveries in chemotherapy could not have been made and notably of arsphenamin and its allied compounds for the treatment of syphilis, frambesia and other diseases; without it chemotherapeutic research would be seriously crippled and rendered all but sterile along with the regrettable rushing of new compounds into the treatment of sick human beings without any preliminary knowledge of their capacity for either harm or good. Furthermore, and properly so, the lower animals themselves have greatly profited by much of this experimental work among them, as several remedies have been found of practical value in the treatment of their diseases, as I hope not to overlook describing in subsequent chapters. While it cannot be denied that empiricism has given us many chemotherapeutic remedies of great value, as discussed above, yet only scientific and well regulated chemotherapeutic investigation can be confidently expected to contribute most advance in the future, of which no small part will be well-conducted animal experiments for determining the possible curative effects of new compounds as they are produced.

Even *in vitro* parasiticidal tests have proved of value, in spite of well-defined limitations and especially in relation to the chemotherapy of bacterial diseases, but the technic of these as well as of trypanosome and spirochetic tests and infections will be described and discussed with more detail in later chapters, as the subjects are too lengthy and important for brief consideration in this place.

Value and Limitations of Toxicological Tests with the Lower Animals in Relation to Chemotherapy.—In this chapter, I wish especially to consider the value and limitations, as well as the methods, for determining the toxicity of chemical agents and drugs for the lower animals in relation to chemotherapy. Without doubt, differences in the physiological functions of such lower animals as white mice and rats, guinea-pigs, rabbits, cats and dogs employed in toxicity tests do not ordinarily permit one to determine all of the toxic qualities of a compound for man, yet the chief toxic effects are readily elicited and a good idea gained of the toxic dose per kilogram of weight which usually yield a safe guide to dosage in administration to man. This has been found true of the organic arsenicals, so that an official rat test for toxicity is required of arsphenamin, neoarsphenamin, sulpharsphenamin,

etc., although rats do not show some of the early or immediate toxic reactions like the "nitritoid crisis," nausea and vomiting, sometimes following intravenous injections of these compounds. Doubtless, this is due in part to the absence of imagination and nervous apprehension on their part which may play an important rôle in the production of apparently toxic reactions in human beings.

Nevertheless, the fact remains that the value of toxicity tests among the lower animals is limited by differences in physiological function and this in turn is influenced by the species employed, age, weight, sex, seasons and diet, as I shall shortly discuss in the following section dealing with methods. For example, the mouse can usually stand more arsphenamin and allied compounds per gram of weight than the rat and both of these more than the rabbit and dog. I believe that this is also true of the organic mercurials and that it can be stated as a general rule that human beings cannot stand nearly as much of these compounds per gram of weight as the rat. In other words, the larger and heavier our test animal, the less is its tolerance, which does not bear a strict relation to body weight.

Furthermore, our test animals are usually normal and healthy which naturally yields a greater resistance to the toxic effects of chemical agents than diseased animals and human beings. Rats infected with *Tr. equiperdum*, for example, frequently cannot withstand as large doses of arsphenamin or neoarsphenamin per kilogram of weight as normal rats. Doubtless, the occurrence of toxic reactions from arsphenamin and allied compounds in human beings is sometimes due primarily to underlying pathological states produced by syphilis or some other disease materially altering the metabolization and excretion of the drug with an increase of toxicity.

But despite the influence of species and racial idiosyncrasy in the lower animals, of size and weight, general conditions of health, diet, sex, etc., experience has shown, as stated by Sollman: "*If a given poison affects all species alike, it may be concluded that its action on man is also the same.* If it produces different effects, but if these can be explained by differences in physiology, the effects on man will be similar to those produced on the species the physiology of which resembles most closely of man."

Maximal Tolerated and Minimal Lethal Doses of Compounds in Relation to Toxicity Tests.—As a general rule, toxicity tests with the lower animals aim to elicit the following data: (1) The largest amount of drug per kilogram of weight compatible with life for a stated period of time and designated as the *maximal tolerated dose* (M.T.D.); (2) the smallest amount of drug per kilogram of weight producing death at once or within twenty-four hours and designated as the *minimal lethal dose* (M.L.D.); (3) whether or not acute symptoms or "early reactions" are produced, and (4) whether or not pathological changes in the tissues are produced and largely responsible for the "late reactions."

All of these and especially the first three, are influenced to some extent by the animal employed, the route of administration, the concentration of the solution of compound, the rate of its injection, if by the intravenous route, and numerous other factors. In other words, the M.T.D. and M.L.D. of any given compound are by no means fixed values and the same or approximately the same figures with the same compound tested in different laboratories are to be secured only by adhering strictly to a standard technic. For these reasons the Hygienic Laboratory of the United States Public Health and Marine Hospital Service has laid down a detailed method for the testing of arsphenamin and allied compounds employed in this country and somewhat similar regulations are in force in England and elsewhere.

As a general rule, the maximum tolerated dose of a compound is, by all odds, the most important value in relation to chemotherapy and is practically always less than the minimal lethal dose. It is determined by giving a series of rats or other animals, of approximately the same age, weight and general condition, single doses of increasing amounts per kilogram of weight of a solution of stated concentration at a stated rate and stated route, to determine the largest amount per kilogram borne for ten days to two weeks by at least 60 per cent. of the animals injected. Duration of life is practically the sole criterion and at least ten days are allowed for the production of late effects; two weeks is a better interval for most compounds and especially for the arsenicals and mercurials. The maximal tolerated dose may be likewise determined by giving a series of administrations at stated intervals over a given period of time, but the results are not as sharp and clear cut as when single doses are injected.

The minimal lethal dose is determined in the same manner and represents the smallest amount per kilogram of weight proving fatal for at least 60 per cent. of animals at once or within an arbitrary period like one to twenty-four hours after administration. As a general rule, only single doses are given, but it is to be emphasized that the figures are of scientific value only when a uniform method is employed, since details, often those appearing but trivial, may exert an important influence upon the results. For these reasons, the data on toxicity of compounds given in subsequent chapters can be regarded only as approximately correct, because they have been based upon quite a variety of different methods employed by many investigators.

Factors Influencing Toxicity and Therapeutic Tests in the Lower Animals. A considerable amount of our information bearing upon toxicity tests in relation to chemotherapy has been gained from studies upon the toxicity of the organic arsenicals, and especially arsphenamin and its congeners, for mice, rats, guinea-pigs and rabbits. Of course, pharmacologists have employed some of these animals, and more especially dogs, for toxicity tests for many years, but the advent of arsphenamin and its allied compounds and the necessity for checking and controlling their toxicity, trypanocidal and spirocheticidal properties, has resulted in a much wider employment of toxicity and parasiticidal tests in laboratories engaged in the preparation of these and other chemotherapeutic compounds.

Choice of Animal.—For pharmacological studies upon the influence of drugs upon the heart and circulation, respiration, etc., the dog would appear to be mostly employed, with the cat or rabbit as second choice. But for routine toxicity, trypanocidal, spirocheticidal and bactericidal tests in relation to chemotherapy, the white mouse (*Mus musculus albus*), white rat (*Mus Norwegicus albus*), guinea-pig and rabbit are usually employed. Ease of handling, costs, cage space, whether multiple intravenous injections are to be given, etc., are factors sometimes influencing the choice.

On the whole, I believe that the *white rat* of 100 to 200 grams weight is the best animal of all for routine toxicity and trypanocidal tests. This is true at least in the testing of arsphenamin, neoarsphenamin and allied compounds in which I have had most of my experience. I have also employed rats in toxicity tests with various dyes, cinchonics, organic and inorganic mercurials and numerous organic compounds of other heavy metals, with uniform satisfaction. Indeed, a rather extensive experience during the past eleven years with toxicity and various parasiticidal tests employing rabbits, guinea-pigs, rats, and mice, has convinced me that the white rat is the best animal of all and especially when a good healthy stock is available.

Influence of Diet and General Care of Animals.—In 1910, Hunt[3] found that the susceptibility of rats, mice, and guinea-pigs to certain poisons was profoundly altered by comparatively slight changes in diet. Lake[4] has stated that rats fed on a well-balanced ration and in a good state of nutrition will tolerate a higher dose of arsphenamin or neoarsphenamin, and react more uniformly than poorly nourished animals. Hooper, Kolls and

Wright,[5] Roth and I have also observed that diet was important in securing constancy in the results of toxicity tests in rats with arsphenamin and allied compounds.

As stated by Lake, the health and general nutrition of rats are important factors in resistance to arsphenamin and in his opinion, variations in susceptibility in rats from different colonies or dealers are due solely to differences in the health and nutrition of the animals. Differences in the toxicity of lots of arsphenamin amounting to as much as 20 per cent. were ascribed to these factors.

Lake also observed that rats after feeding may be 10 per cent. heavier and that if used for toxicity tests, may receive an excessive dose according to body weight. He also found them somewhat more susceptible to arsphenamin after a meal, which is borne out by clinical experience showing that patients are more liable to react on a full stomach. For these reasons, rats should not have access to food for at least twelve to eighteen hours before being injected with any compound.

Rats breed rapidly, are clean and easily kept clean, inexpensive to maintain in large numbers, easy to handle and comparatively easy to treat by any route of administration. Donaldson[6] has published a most interesting and instructive book on this valuable laboratory animal containing information on its life history, feeding, watering, general care, etc. Osborne and Mendel[7] state that partly colored rats may give inconstant results; my experience agrees with that of Roth,[8] that they are generally more resistant to arsphenamin and neoarsphenamin than those entirely white. The diet of rats may consist of white bread, cow's milk, cracked corn, oats, and, in addition, twice weekly, fresh beef and cabbage, or spinach, carrots, etc. Further suggestions in their feeding and care have been made by King,[9] Slonaker[10] and Ferry.[11]

White mice may be given a diet quite similar to that of rats, consisting of a liberal supply of cow's milk, white bread, and a mixture of cracked corn, oats, and sunflower seeds with plenty of fresh water.

The care of the *guinea-pig* differs slightly from that of the mouse and of the rat. The instructions issued by Lantz[12] state that improper, irregular, and deficient feeding are common causes of inflammation of the stomach and intestines, from which losses among the animals may be very great.

Influence of Age, Weight, Body Surface, Blood Volume and Metabolism of Animals.—As shown by Roth[13] in experiments bearing upon the influence of the age and weight of white rats upon the toxicity of arsphenamin and neoarsphenamin administered by intravenous injection, animals weighing from 73 to 98 grams were more resistant than animals weighing from 180 to 275 grams, and largely on the basis of his investigations the Hygienic Laboratory requires that rats employed for toxicity tests with the arsenicals shall weigh from 100 to 150 grams in order to minimize variations due to these factors. According to Donaldson, albino rats which have developed normally and which range in weight between 100 and 150 grams, are from two to five months old. Donaldson's figures from the Wistar Institute colony show that at two months of age, the males averaged 123 grams and the females 107 grams, while at three months of age the males averaged 185 grams and the females 148 grams. On the other hand, the figures which he gives for the New Haven Colony of the Connecticut Agricultural Experiment Station show that at two months of age the males averaged 97 grams and the females 89 grams; at three months of age the males in the same colony averaged 144 grams and the females 115 grams, while females which averaged 150 grams were 150 days old (five months). From the above data, it would appear that normal rats which range in weight from 100 to 150 grams are at least two months and not over five months old, and that a female of 150 grams is considerably older than a male of corresponding weight. Both males and females are sexually mature at two months of age and growth is quite rapid about this period. It is thus seen that the Hygienic Laboratory test requires that the rats used for the special tests on arsphenamin shall be young, sexually mature, and in an active period of development.

Hammett and Nowbrey[14] also found that with advancing age there is a progressive diminution in the resistance of the albino rat to death from arsenic poisoning. A dose of 11 mgm. or over of arsenic trioxid is fatal to young rats of 60 to 90 days of age. By the time the animals have reached the age of 120 to 150 days a dose of 9 mgm. is fatal. When they are 210 to 240 days old, 8 mgm. is the amount which produces death within twenty-four hours in the average animal.

My own results have not shown very striking differences in toxicity tests with arsphenamin and neoarsphenamin, insofar as rats weighing from 90 to 190 grams were concerned. For example, of 358 rats weighing from 90 to 125 grams, injected intravenously with arsphenamin (0.100 gm. per kilo) in 2-per cent. solution at the rate of 1 c.c. in two minutes, 213 or 59 per cent. survived forty-eight hours; of 131 rats weighing from 130 to 150 grams, 94 or 71 per cent. survived; of 37 rats weighing from 160 to 180 grams, 25 or 67 per cent. survived. Of 399 rats weighing from 90 to 125 grams and receiving intravenous injections of neoarsphenamin (0.200 gm. per kilo) in 4-per cent. solution at the same rate, 314 or 78 per cent. survived seven days; of 147 rats weighing from 130 to 150 grams, 116 or 78

per cent. survived seven days and of 46, weighing from 160 to 190 grams, 36 or 78 per cent. survived. Therefore, differences in age and weight influenced tolerance for arsphenamin to a greater extent than tolerance for neoarsphenamin.

Of course, age and weight of human beings greatly influence dosage in chemotherapy, as I shall discuss shortly, but here it is of interest to inquire into the mechanism and nature of the variations due to these factors. Hammett and Nowbray have expressed the opinion that differences in resistance of man and rat to arsenic can be attributed in large part to a difference in the respective rates of protein metabolism. With advancing age, the active mass of protoplasmic tissues and blood volume decrease and probably signify a decreased capacity for dealing with toxic agents. Dreyer and Walker [15] state that the concentration of drugs and other foreign substances distributed in the blood plasma is proportional to the body surface, since blood volume is proportional to the body surface according to the formula $B = W\frac{n}{k}$, where n is approximately 0.72, and k is a constant to be ascertained for each particular species of animal. The same apparently applies to internal surfaces, such as that of the alimentary canal, since Moore[16] came to the conclusion that, in regard to drugs, tolerance was limited by the surface area of the mucous membrane of the alimentary canal, offering a valid explanation of the fact that it is often difficult or impossible to administer effective doses of drugs to large animals, since they do not tolerate doses required to produce the same concentration in their blood as is needed for successful therapeutic action in small animals. Dreyer and Walker, after analyzing the toxic doses of arsenicals, toxins, and other substances, have reached the conclusion that in animals of different sizes in any given species, the dose required to produce a given effect is related to the body surface and blood volume of the animal and not directly to the body weight; that is to say, smaller individuals require a relatively larger dose than the heavier animals to produce the same effect. They believe it follows that in the accurate measurement and standardization of toxic substances and antitoxins, it will now be possible to make use of animals of different weights within a given species, instead of using only animals of an arbitrarily selected weight. The influence of age, weight, blood volume, etc., upon the parasiticidal activity of chemical compounds has not been the subject of as much interest and investigation. In my experiments with *Tr. equiperdum* infections of white rats varying in weight from 80 to 260 grams, no constant variations in the trypanocidal effects of arsphenamin and neoarsphenamin have been noted. I believe, however, that age and weight of rabbits may influence the results of spirocheticidal tests, but largely because these and related factors influence the susceptibility and resistance of these animals to *Sp. pallida*. In other words, age, weight and related factors may influence the course of an experimental infection by bringing into play variations in immunological resistance, but they do not appear to appreciably influence directly the parasiticidal activity of chemotherapeutic agents. While the rabbit and guinea-pig infected with virulent trypanosomes are more difficult to cure than infected mice and rats, yet this does not involve the factors of age and weight, but, rather, the subject of variations due to animal species.

Influence of Species and Individual Variation.—In other words, results in dosage calculated for one species of animal cannot directly be applied to another species merely by taking surface and blood volume into consideration, since tolerance and intolerance are specific characters shown to be in many cases independent of the size of the species concerned. This factor limits, therefore, the usefulness of data on the toxicity of drugs for the lower animals as applied to human beings. I have already mentioned that tolerance of the rabbit for the arsenicals is appreciably less than for the rat as reported by Schamberg, Raiziss and myself,[17] and I believe this is due more to variation in specific tolerance than to age, weight, body surface, blood volume or variations in metabolism. Clinical experience also indicates that the tolerance of human beings as a class for the arsenicals is less than that of mice and rats and probably more closely approaches that of the dog and rabbit.

Individual variations in tolerance are also sometimes striking and not to be explained on these factors of body surface, blood volume and rate of metabolism. They are encountered not only among human beings, but also among rats and rabbits, as reported by Roth,[18] Lake and others. No matter how uniform the stock of rats, or how carefully they are selected, it is impossible to secure uniformity of results within narrow limits, without using a considerable number of animals with the same dose. For this reason it is almost impossible to define sharply the maximum tolerated dose of a compound per kilogram of weight, but we must rather express it as the maximum dose allowing a majority of animals to survive for a stated period of time.

Influence of Sex of Animals.—According to Lake, *pregnancy* of albino rats tends to reduce their resistance to arsphenamin. Since the pregnant uterus of these animals may constitute as much as 10 to 25 per cent. of the entire body weight, it is obvious that if such an animal receives a dose based on body weight, the maternal blood will contain a relatively

larger amount of the drug than that of a non-pregnant animal of the same weight. There is also the possibility that pregnancy itself affects susceptibility in some degree, although we have no data on this point. Since it is difficult to determine pregnancy in a rat from the appearance of the animal alone, unless the condition is moderately advanced, it is important to conduct a necropsy on every animal succumbing, and in case pregnancy appears to have influenced the result, the test should be repeated. Rats of 100 to 120 grams in weight are only occasionally found pregnant, but the most satisfactory method for avoiding the difficulty consists in separating the females from the males, when they have reached a weight of 80 to 90 grams.

Otherwise, sex does not appear to appreciably influence the tolerance of the lower animals for chemical agents. My own experience has been of a similar character, but if differences do exist, the non-pregnant female rat is probably slightly more resistant than the male of similar weight; for example, of 327 male and 325 non-pregnant female rats of 95 to 150 grams used in testing the toxicity of 71 different lots of arsphenamin in dose of 0.100 gm. per kilogram, 76 per cent. of the males survived and 85 per cent. of the females; of 432 males and 320 females of similar weight range used in testing the toxicity of 66 different lots of neoarsphenamin in dose of 0.200 gm. per kilogram, 79 per cent. of males and 84 per cent. of females survived.

Influence of Seasons.—Hunt[19] has observed that guinea-pigs are most resistant to acetonitril in the spring and summer months whereas mice were least resistant at those times. Roth[20] found that rats were more resistant to neoarsphenamin during the summer than during the winter months.

Influence of Marking and Handling Animals.—While rabbits and guinea-pigs are usually identified by metal tags or by registration of their color, mice and rats are generally marked by dyes on the tail or body or by clipping the fur. I have long used a saturated alcoholic extract of gentian-violet for this purpose with uniformly satisfactory results. In the Hygienic Laboratory a solution of picric acid has been employed, but after giving this method a trial, I became convinced that it increased the death rate among rats and mice employed for toxicity and trypanocidal tests with arsphenamin and neoarsphenamin. This appeared to be due to licking of the stains by the animals and possibly to some extent from absorption through the skin. Roth[21] has recently stated that he has been led to conclude that picric acid acted as a synergist to arsphenamin when mice were used as test animals, that is to say, increased the toxicity. For this reason, he advised the practice of clipping the animals over certain areas, but I have found marking with gentian-violet much less time consuming and quite satisfactory.

Lake states that the method employed in holding animals, which causes the least nervous irritation and physical exertion during the administration of the drug, has an important influence on lowering the toxicity. Since changing his method of holding rats by hand to my method of tying them to a board, shown in Fig. 1, the number of deaths occurring within a few minutes after the intravenous injection of arsphenamin has decreased to a marked extent. Of course, much less influence is exerted upon the results of trypanocidal tests, since the doses administered are smaller than employed in toxicity tests.

Influence of Route of Administration of Drugs.—As a general rule, the toxicity of drugs for the lower animals is determined by intravenous, intraperitoneal, intramuscular or subcutaneous injection. Occasionally, such tests are conducted by oral administration, subthecal or intrapleural injection, *but the route of administration has a profound influence upon toxicity and tolerance. It should be a general principle to administer a compound to the lower animals for toxicity and parasiticidal tests, by the same route or routes as are to be employed for administration to human subjects in the treatment of disease.* For example, arsphenamin is now administered almost entirely by intravenous injection and it is proper to test its toxicity and parasiticidal activities by the same route; sulpharsphenamin, however, is given by subcutaneous, intramuscular or intravenous injection and it is proper to determine its toxicity and parasiticidal (trypanocidal or spirochaeticidal) activities by intravenous and subcutaneous injection, since both values vary according to these routes of administration. To test the toxicity of a drug like arsphenamin by subcutaneous injection in mice or rats is a mistake; the compound should always be tested by the same route of administration as is used in its practical administration in order to elicit the considerable influence exerted by the blood and other factors upon toxicity. Route of administration is less important in relation to parasiticidal activities, but even here the influence is not inconsiderable or to be neglected.

In a general manner, intravenous injection produces a higher degree of toxicity than either subcutaneous or intramuscular injection or oral administration; an exception to this general rule, however, is noted with neoarsphenamin, which curiously, is not as well borne by subcutaneous injection as by intravenous and intraperitoneal injection. This was first described by Castelli[22] and subsequently confirmed by myself.[23]

Schamberg, Raiziss, Trist and I[24] have found the average relationship between intravenous and intramuscular injections of mercurial compounds to be about 4 to 1. For the mouse Castelli found arsphenamin 1.7 times less toxic by subcutaneous than by intravenous injection and neoarsphenamin about 10 times more toxic by subcutaneous than by intravenous injection. Schamberg, Raiziss and I[25] found arsphenamin about 3 times less toxic for the white rat by subcutaneous injection than by intravenous injection and neoarsphenamin about 0.6 to 0.7 times more toxic by subcutaneous than by intravenous injection. There was no constant or uniform relation among the highest tolerated doses of these different compounds for rats with the same route of administration. In very general terms, a compound is apt to be about 10 times more toxic by subthecal than by intravenous injection; 0.6 to 0.7 times more toxic by intrapleural, 1 to 4 times less toxic by intraperitoneal and 2 to 16 times less toxic by subcutaneous, than by intravenous injection.

Influence of Concentration of Solutions of Drugs and Rate of Injection upon Toxicity and Parasiticidal Tests.—These two factors are so closely related that they may be considered together. The rate of injection may be varied, while the concentration of the solution remains constant, or the concentration varied while the rate remains constant, so that the same amount of drug is mixed with the blood in the same length of time.

In the experiments of Schamberg, Raiziss and myself with solutions of arsphenamin varying in concentration from approximately 1 to 16 per cent. and slowly administered to rabbits by intravenous injection with a syringe, it was observed that increasing the concentration did not augment toxicity when *slow* injections were given. Subsequently I observed the same results with neoarsphenamin and sulpharsphenamin, but with all compounds, toxicity may be markedly increased by rapid injection and especially in the case of arsphenamin. For example, Lake has reported that of a series of rats injected intravenously with 0.060 gm. of arsphenamin per kilo in 1- or 2-per cent. solution, 75 per cent. died within four hours after rapid injection, while with the less rapid rate only 16.6 died. In all toxicity tests, therefore, these factors of rate of injection and concentration of solution require standardization and in the Hygienic Laboratory method for testing the toxicity of arsphenamin and neoarsphenamin, it is required that the former in 2-per cent. and the latter in 4-per cent. solution shall not be injected intravenously at a rate exceeding 0.5 c.c. per minute.

These experimental results are in keeping with clinical experience and especially in relation to the administration of arsphenamin. Neoarsphenamin and sulpharsphenamin may be injected in 5- to 10-per cent. solutions (0.6 to 0.9 gm. in 10 to 20 c.c.) and even in 30-per cent. solution (0.6 gm. in 2 c.c.) by means of a syringe without evidences of increased toxicity when given *slowly*, but the toxicity of arsphenamin is much more likely to be increased by such methods of administration, as discussed with more detail in Chapter LXII.

Period of Observation in Relation to Toxicity and Parasiticidal Tests in Animals.—Finally the period of time with which animals are kept under observation in toxicity and parasiticidal tests is one of considerable importance. Some drugs and chemical compounds show *early* toxic reactions, of which arsphenamin is a type; others show *delayed* toxic reactions, of which neoarsphenamin is a type, and when one reports upon the minimal lethal or maximum tolerated doses of any compound it is necessary to define the period of time arbitrarily adopted for determining the results. From the standpoint of the official Hygienic Laboratory rat test for the toxicity of the arsenicals, Roth has stated that it is apparent that when arsphenamin is examined one tests for its "acute toxicity" principally, since the rats die within forty-eight hours, for the most part within twenty-four hours. Rats, therefore, furnish no definite information as to the possible late effects of arsphenamin. However, rats show immediate symptoms after unusually toxic preparations of arsphenamin are given in the official dosage. These symptoms may probably be taken in part as a measure of the state of combination of the arsenical. Neoarsphenamin, on the other hand, causes slight or no immediate symptoms in rats on the official dosage, even when the drug has become insoluble; furthermore, the death is "subacute" or delayed, the animals dying, as a rule, after forty-eight hours. Mice die from "acute poisoning" after both arsphenamin and neoarsphenamin and, therefore, give a limited view of the behavior of neoarsphenamin in the organism. Guinea-pigs, like rats, usually die from "acute poisoning" after arsphenamin and from "delayed poisoning" after neoarsphenamin.

The results of my tests substantiate these observations. For example, of 201 different lots of arsphenamin tested by intravenous injection in dose of 0.100 gm. per kilo in 1042 rats according to the Hygienic Laboratory method, and 280 different lots of neoarsphenamin tested in dose of 0.200 gm. per kilo in 1585 rats, the death rates were as follows:

	Arsphenamin (Per Cent.)	Neoarsphenamin (Per Cent.)
At once	11.1	0.63
At end of 24 hours	15.4	3.2
At end of 48 hours	3.2	1.9
At end of 3 days	0.57	4.2
At end of 5–7 days	2.3	17.6
At end of 8–10 days	2.3	4.4
At end of 12–14 days	2.2	2.9
Surviving more than 14 days	62.2	64.0

In other words, about 38 per cent. of the rats injected with arsphenamin finally succumbed, but about 30 per cent. of these deaths occurred within forty-eight hours after injection and only 8 per cent. at subsequent periods. About 36 per cent. of the rats injected with neoarsphenamin succumbed, but only 5.7 per cent. in forty-eight hours, while 27.5 per cent. perished in seven days.

Somewhat similar results have been observed in a different set of tests. Of 181 lots of arsphenamin tested in doses varying from 0.100 to 0.200 gm. per kilo and of 278 lots of neoarsphenamin tested in doses varying from 0.200 to 0.400 gm. per kilo, the highest average doses producing death of the rats at varying intervals were as follows:

	Arsphenamin (Gm. per Kilo)	Neoarsphenamin (Gm. per Kilo)
At once	0.150	0.367
Survival 1 day	0.133	0.343
Survival 2 days	0.129	0.340
Survival 5 days	0.128	0.318
Survival 7 days	0.128	0.317
Survival 10 days	0.128	0.316
Survival 14 days	0.127	0.315

In other words, the highest tolerated dose of arsphenamin for rats remains quite constant after the first two days following intravenous injection, but with neoarsphenamin a decided drop occurs on or about the fifth day, following which the tolerance remains quite constant. Arsphenamin, therefore, produces acute or early toxic reactions, while neoarsphenamin produces subacute or late reactions; for these reasons, the Hygienic Laboratory permits reporting upon the maximum tolerated dose of arsphenamin at the end of forty-eight hours after injection, but requires a period of observation of seven days in the case of neoarsphenamin.

In trypanocidal tests by my method described in Chapter X, rats are kept under observation for at least two weeks because trypanosomiasis may develop as late as ten to fourteen days after inoculation when arsphenamin or some other trypanocidal agent is given in a dose just short of the completely sterilizing dose. In Voegtlin's method, however, the results are evaluated in two days after injection of the drug, but sometimes the blood of a rat showing no trypanosomes at this time will subsequently develop trypanosomiasis and perish. I believe, therefore, that in trypanocidal tests the animals should be kept under observation sufficiently long to express the minimal curative dose of the compound under study, that is to say, the smallest amount of drug per kilogram of weight capable of completely sterilizing the majority of a series of rats.

In spirocheticidal tests employing rabbits, a much longer period of observation is required, as described in Chapter XXI. Merely to determine the approximate amount of compound per kilogram of weight capable of removing spirochetes from scrotal or testicular lesions is insufficient; the neighboring lymphatic glands must be removed and inoculated into the testicles of additional rabbits to determine whether or not *complete* sterilization has been accomplished. Quite a number of compounds may cause spirochetes to disappear from the scrotal and testicular syphilitic lesions of rabbits, because these tend to heal spontaneously anyhow, but complete sterilization of the lymph-glands is another and very important matter, requiring at least four to six months of time for a complete test.

Factors Influencing the Dosage, Toxicity and Therapeutic Activity of Compounds in Man.—A considerable amount of the data to be secured by properly conducted toxicity tests with the lower animals is directly applicable to human beings. From the standpoint of chemotherapy, much of our data concerns arsphenamin and neoarsphenamin, but the principles established by experience with these is apparently applicable to other drugs and compounds administered after the same methods. Unquestionably

such factors as the diet, general health, individual susceptibility, pregnancy, route of administration, concentration of solutions, and rate of injection bear an even more important relation to the toxicity of drugs for human beings than they do for rats and other lower animals, as I shall discuss with considerable detail in subsequent chapters, but it is surprising how closely the data secured by animal tests corresponds to clinical experience. For these reasons, animal tests for the toxicity and parasiticidal activity of chemotherapeutic compounds are of great value when properly conducted and evaluated despite well recognized limitations and deficiencies.

I may, however, discuss at this time the matter of *dosage in relation to age and body weight.* In the first place children appear to stand better than adults such chemotherapeutic compounds as arsphenamin, neoarsphenamin, sulpharsphenamin, quinin, mercury and bismuth when based upon weight; as stated by Fowler[26] in relation to the solution of arsenic bearing his name, "I observed that children were less apt to suffer from the medicine than adults."

Age probably influences the toxicity and dosage of drugs by variations in blood volume, body surface and rate of protein metabolism. In the young the metabolic rate is greater than in that of adults, as has been indicated by Benedict[27] in his studies of the energy requirements of children. Furthermore, as shown by Benedict and Talbot,[28] there is some evidence that the active mass of the protoplasmic tissue determines the fundamental metabolism, and with advancing age this mass decreases in relation to the organism as a whole. Moreover, the blood volume decreases with advancing age, and as previously mentioned, Dreyer and Walker have shown that drug tolerance bears a close relation to this factor and body surface. These authors state that dosage in relation to the surface works out very simply as follows:

At 21 years	Full dose	At 3–4 years	$\frac{1}{3}$ dose
At 15 years	$\frac{3}{4}$ dose	At 1 year	$\frac{1}{4}$ dose
At 9–10 years	$\frac{1}{2}$ dose	In the early months	$\frac{1}{10}$ dose

To the best of my knowledge, age *per se* does not influence the parasiticidal activity of chemotherapeutic compounds. At least trypanosomiasis in a rat approximately three years old, which, according to Donaldson may be regarded as corresponding to a man ninety years old, is not more difficult to cure with arsphenamin or neoarsphenamin than a rat thirty-one days old which corresponds to a child of about two and one-half years. Testicular syphilis of old rabbits is likewise cured by these compounds as readily as similar lesions of the same duration in young rabbits. The duration of a disease profoundly influences parasiticidal effects, but the age of the patient probably does not and certainly not to the same degree as susceptibility or tolerance to toxic effects of drugs.

Technic of Toxicity Tests with the Lower Animals, with Special Reference to Arsphenamin and Neoarsphenamin.—It may be well to describe here with some detail the official Hygienic Laboratory method for determining the toxicity of arsphenamin, neoarsphenamin, sulpharsphenamin and allied compounds, because the method takes into consideration all of the factors influencing the results of toxicity tests in animals and serves as an excellent example of a technic applicable to the determination of the toxicity of other compounds. At least, I have found this method as herein described perfectly satisfactory for testing the mercurials, dyes, cinchonics and various organic and inorganic compounds of the heavy metals prepared in the course of chemotherapeutic investigations; some compounds may demand minor modifications in technic, but the method as a whole is an excellent one.

Plea for Standardized Methods.—The need for a standardized technic is evident when one considers the different methods employed for testing the toxicity of arsphenamin and neoarsphenamin in other countries as England, Canada, France and Japan, as recently reviewed by Roth.[29] For example, as there is no governmental regulation in Germany over the quality of drugs manufactured or sold in that country, the regulation of arsphenamin and neoarsphenamin is left entirely in the hands of the manufacturers. As a result of the various methods in use, a great deal of confusion exists in reference to the maximal tolerated doses of various compounds of chemotherapeutic interest and I wish here to enter a plea for the adoption of a standardized method for determining the toxicity of compounds for which I would propose the Hygienic Laboratory method of the U. S. Public Health and Marine Hospital Service, since it is suitable not only for testing the arsenicals, but for most other compounds as well.

Hygienic Laboratory Method for Testing the Toxicity of Arsphenamin.—The method employed in this country and largely built upon the studies of Schamberg, Raiziss and myself, Gies, Lake and Roth, is as follows:[30]

(1) The total arsenic content of the drug as obtained from the ampule shall not be below 30 *per cent.* nor above 32 *per cent.*, when determined according to Lehmann's method.

(2) The maximum tolerated dose shall not be less than 120 milligrams per kilo body weight for albino rats when tested as follows:

(3) Healthy white rats weighing from 100 to 150 grams are employed. Pregnant animals shall not be used. The diet may be of white bread, cracked corn, oats, cow's milk, and, in addition, twice weekly, fresh beef and cabbage or some other vegetable, but no food is given for at least 12 to 18 hours before the tests, though water should be supplied during this time.

(4) The drug shall be dissolved in freshly glass-distilled water and made into the alkaline solution by the addition of 0.9 c.c. $\frac{N}{1}$ sodium hydrate for each 0.1 gm. of the drug. The final concentration of the drug shall be in the proportion of 2 parts to 100. The drug shall be completely soluble in water in this proportion.

(5) Injection should be made into the saphenous vein by means of a burette or a syringe, accurately graduated to 0.01 c.c. When hemorrhage occurs it should be controlled mechanically.

(6) The rate of injection shall be at from 12 to 15 seconds for each 0.1 c.c. of the solution.

(7) An anesthetic is not required.

(8) For each toxicity test a series of not less than five rats shall be used, and at least 60 per cent. of the animals injected must survive at least forty-eight hours from the time of the injection, provided that, if the first test is made on five rats only and more than one die, the test must be repeated and the final result based on the total number of rats injected.

Hygienic Laboratory Method for Testing the Toxicity of Neoarsphenamin.—This is conducted in exactly the same manner with the following specifications:

(1) The total arsenic content of the drug as obtained from the ampule shall not be below 18 nor above 20 per cent. when determined according to Lehmann's method. The drug should be freely mobile in ampule and soluble in cold water (1 gm. in 10 c.c. of water in 5 minutes).

(2) The maximum tolerated dose shall not be less than 240 milligrams per kilo body weight for albino rats.

(3) The drug shall be dissolved in cold glass-distilled water. A preparation which is not readily soluble in cold distilled water should be discarded at once as a dangerous product. The final concentration of the solution shall be in the proportion of 4 parts per 100.

(4) For each toxicity test a series of not less than five rats shall be used, and at least 60 per cent. of the animals injected must survive at least seven days from the time of the injection, provided that if the first test is made on five rats only and more than one die the test must be repeated and the final result based on the total number of rats injected.

Hygienic Laboratory Methods for Testing the Toxicity of Sulpharsphenamin, Silver Arsphenamin, Silver Neoarsphenamin and Sodium Arsphenamin.—These are conducted in the same manner. Each must contain at least 20 per cent. of arsenic when determined according to Lehmann's method, with plus or minus 1 per cent. The solutions are prepared in the same manner as solutions of neoarsphenamin and the concentration of each drug shall be in the proportion of 4 parts in 100.

The maximum tolerated dose of *sulpharsphenamin* shall be not less than 300 mgm. per kilo bodyweight for albino rats; the dose for *silver arsphenamin* shall be not less than

140 mgm.; *silver neoarsphenamin* not less than 240 mgm. and *sodium arsphenamin* not less than 180 mgm. per kilo of body weight.

For each toxicity test a series of not less than five rats shall be used, and at least 60 per cent. of the animals injected must survive at least 7 days from the time of the injection, provided that if the first test is made on five rats only and more than one die the test must be repeated and the final result based on the total number of rats injected.

Apparatus for Intravenous Injection of Rats.—While rats may be injected at the uniform rate of 0.5 cc. in 60 seconds by means of a suitable syringe and needle as employed in the Hygienic Laboratory, I have found that these injections are very satisfactorily given in a saphenous vein exposed by a

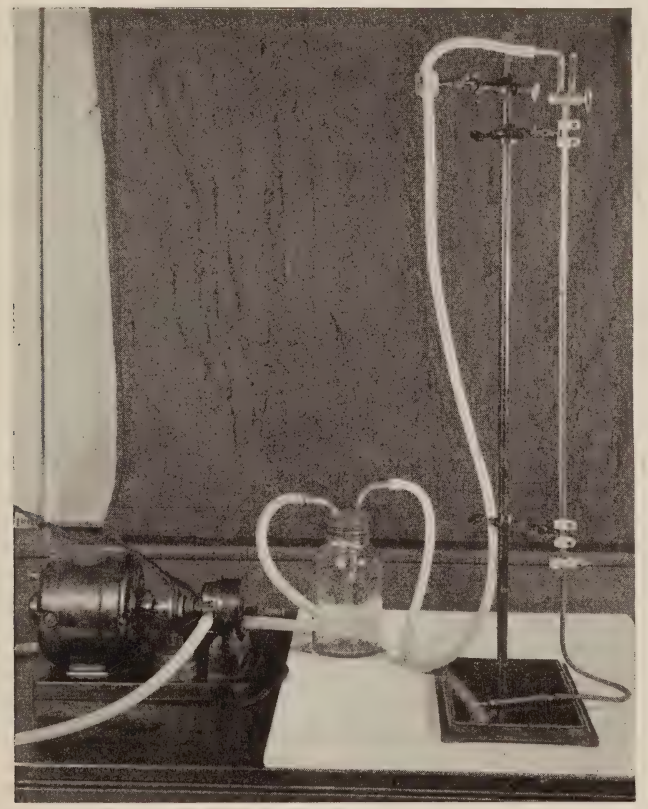

FIG. 1.—BURETTE FOR INTRAVENOUS ADMINISTRATIONS TO ANIMALS.

small incision by means of a gravity method employing the special apparatus designed by me after the apparatus used by Dr. Lake in the Hygienic Laboratory. This apparatus (Fig. 1) is composed of a 2 c.c. burette divided into 0.01 c.c. and fitted with a two-way cock at the upper end for filling by means of suction by vacuum and a water-tight cock at the lower end for stopping the injection. A long glass nozzle is attached to the burette fitted with a short piece of best grade rubber tubing carrying a window near the end, and a needle of No. 26 to No. 22 gauge.

Before injecting the solution the needle and rubber tubing are sterilized by boiling and the burette cleansed by copious flushing by means of the vacuum suction with sterile water followed by the solution to be injected, the water and solution of arsphenamin collecting in the bottle interposed

between the vacuum and the burette. When solutions are changed the apparatus is cleansed between each in the same manner.

After the animal is bound upon the operating board, the skin of the anterior surface of the thigh is cleansed with alcohol and a small incision made over the vein, which is rendered prominent by pressure made in the inguinal region by an assistant. The vein is grasped with fine forceps, due care being taken not to produce pain by grasping the nerve alongside of the vein, the needle is inserted and the cock gradually opened to regulate the flow while the time in seconds is called off by an assistant, until the dose to be given, previously marked off by the rider fastened to the burette, is injected.

With this apparatus it is possible to inject a given amount of solution very accurately and at a given rate of flow set down by the Hygienic Laboratory at 0.5 c.c. per sixty seconds; the amount of solution to be injected is calculated according to the dose to be given per 100 gm. of weight and the rate of injection can be nicely regulated by means of the stop cock and timed with a stop-clock. The apparatus is to be recommended for the intravenous injection of rats, guinea-pigs and rabbits, when a definite rate of flow is required in the conduct of toxicity tests with arsphenamin, tuberculin and various other drugs.

Technic of Intravenous Administration to Mice, Rats, Guinea-pigs and Rabbits.—In conclusion, it may be worth while to describe here the technic of intravenous administration to small laboratory animals; although quite easy for the experienced operator, the technic is usually rather difficult for the beginner and it is hoped that the somewhat detailed accounts given here may be of assistance. Roth[31] has described the following methods for the mouse, rat and guinea-pig:

White Mouse.—The lateral veins of the tail of the white mouse have been found best suited for intravenous administration purposes. The tail must be free from localized or generalized thickening of the epidermis so as to permit the ready entrance of a No. 23, B. & S. gauge needle. The use of a rather long needle, 1 inch in length, was found to be essential; it does not bend easily and therefore can be directed forward more readily than a smaller one.

A mouse weighing between 15 and 20 grams practically always possesses a soft, pliable tail which can be used without any preparation. When a mouse weighing over 20 grams is used, the lateral veins of the tail are usually covered with rather dense tissue, which precludes their use unless the tail is immersed for about a half minute in warm water (about 50°C.). This procedure both softens the skin and dilates the underlying vessels so that the vessels may be successfully used.

For holding the mouse, a small tin mailing tube attached to an iron stand is employed. One end of the metal mailing tube is fitted with a cork having at the circumference a V-shaped opening, which will admit the tail. The other end of the tube contains several small openings for the purpose of admitting air.

The mouse is grasped by the tail with the thumb and forefinger of the left hand and placed in the above-described metal mailing tube, and the cork is inserted so that the tail protrudes through the V-shaped opening. The tail is now straightened by gentle but firm traction and without twisting. The dorsal vein should then appear above, and each lateral to the left and right, respectively.

The syringe, usually a 1-cubic centimeter, all-glass, tuberculin type, graduated to $\frac{1}{100}$ of a cubic centimeter, is balanced between the first and middle fingers of the right hand, the hand resting on the little finger; the thumb is thus free to operate the piston of the syringe. With the syringe held

nearly parallel to the tail, the needle is pushed through the skin over one of the lateral veins (usually the left) and then anteriorly and downward into the vein. If an entrance into the vessel is not effected, either raising or lowering the point of the needle while advancing it further will usually succeed in locating the lumen of the vessel.

White Rat.—The official method for standardizing arsphenamin and allied compounds requires that either the right or left saphenous vein of the white rat shall be employed for the intravenous administration of these compounds. At the Hygienic Laboratory, however, the right vein is more frequently employed, and the procedure is essentially as follows: The animal is tied securely by the legs, back downward, to a flat operating board, by means of strings long enough to permit the hind legs to be lifted easily.

FIG. 2.—ROTH'S METHOD FOR INTRAVENOUS ADMINISTRATION OF THE WHITE MOUSE.

The operating board used in the Hygienic Laboratory is exceedingly simple, consisting of a piece of flat board having the dimensions 18 inches by 8 inches by 1 inch. At the end of the board to which the head is tied are two glass pegs about 1 inch long, set in at an angle in order to hold the string which is looped over the front legs of the animal. Nails in the other end of the board receive the strings which are looped to the hind legs. (Fig. 3.)

After shaving the hair over the skin area covering the left saphenous vein, the left foot is grasped between the third and middle fingers of the left hand, and an incision about one-fourth to one-half of an inch long is made about one-fourth of an inch to the left of and parallel to the vein. The skin is then rolled over to the right with the first finger of the left hand by drawing the skin on the back of the leg to the left. This will bring the vessel into view.

An assistant then makes compression to dilate the vessel. If a syringe is used, it is preferable to employ a 1-cubic centimeter, all glass, tuberculin type, graduated to $\frac{1}{100}$ of a cubic centimeter and fitted with a No. 26 B. & S. gauge needle, five-eights of an inch in length. The syringe is balanced between the first and middle fingers of the right hand, the hand resting on the little finger; the thumb is thus free to operate the piston of the syringe. The needle is then passed through the fascia and upper surfaces of the muscles, about one-eighth of an inch to the left of the vein and almost parallel to it. Advancing the needle slightly farther, the direction is changed so that the needle will enter the vein from the side. After the injection is made, the skin, which was pulled to the right to permit the vessel to come into view, is released,

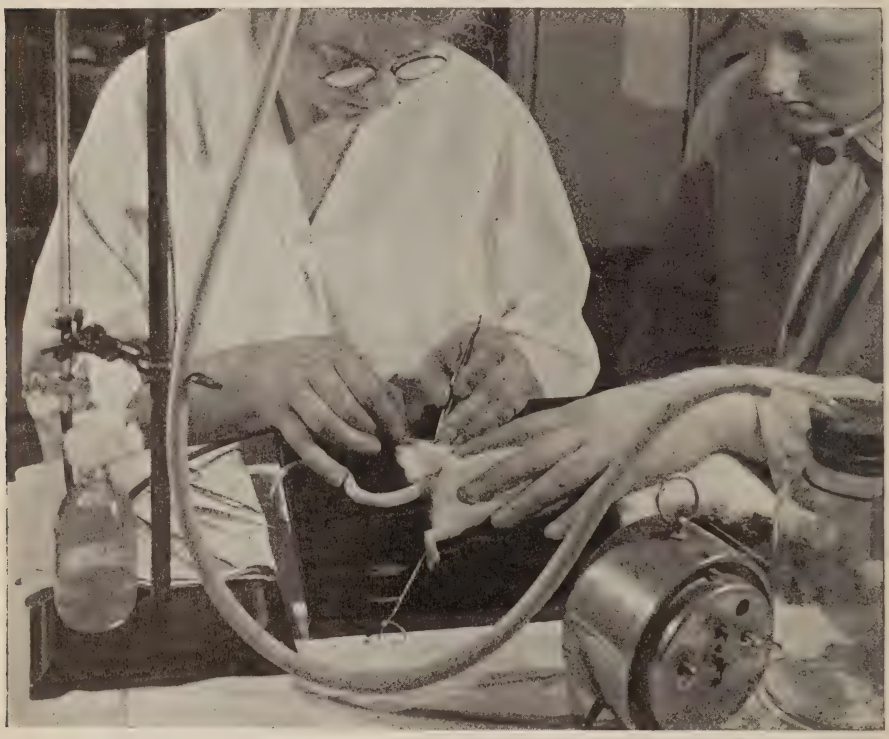

FIG. 3.—METHOD FOR INTRAVENOUS ADMINISTRATION OF THE WHITE RAT IN TOXICITY TESTS.

and this skin flap and the muscles act as effective mechanical checks to hemorrhage, which is quite profuse if the needle is inserted directly into the vein.

If a burette is employed and the injection made by gravity instead of by means of a syringe, a flexible rubber tube is attached to the burette, while the other end of the tube carries a glass tube which is drawn out and ground to fit a No. 23 B. & S. gauge needle 1 inch long. The glass tube is handled in the same way as the syringe, and the vessel is entered in the same manner as was described under the syringe method.

Guinea-pig.—The large superficial vein lying on the dorsal and inner aspect of the hind leg of the guinea pig is well adapted for intravenous administration. Occasionally, however, the vessel may run anteriorly. To use

the above-described vessel for intravenous administration, a special operating board is required. The board proper is similar to an ordinary animal board, except that the end to which the hind legs of the animal are tied has a U-shaped piece cut from it. The board is mounted near the center on an extension shaft which is fitted with two joints, the one at the end to which the board is attached being a ball-and-socket joint and the other an adjustable swivel joint. The shaft is screwed into a metal base which has sufficient weight to hold the board steady when placed in any position. A single operating board, however, may be used as shown in Fig. 4.

The procedure for making the injection is as follows: With the board properly placed in a horizontal position, the animal is tied to it securely, abdomen downward, by means of strings. The board is then placed in a vertical position and rotated on its vertical axis slightly so as to bring the dorsal aspect of the right hind leg into view. After clipping the hair from the leg and shaving it, the leg is lifted up slightly by the first or first and second

FIG. 4.—ROTH'S METHOD FOR INTRAVENOUS ADMINISTRATION OF THE GUINEA-PIG.

fingers and the vein dilated by suitable compression. The vessel can now usually be seen through the skin. A small incision, usually about one-fourth of an inch long, is made diagonally across the leg from the outer lower to the upper and inner aspect, but a trifle to the left of the vessel. The subcutaneous tissue is then pushed aside with a fine pointed forceps, thereby permitting the vessel to come into view.

The vessel is then entered directly or in the same manner as has been described for the rat—that is, by passing the needle of the tuberculin syringe through the fascia and muscles to the left of the vessel and then entering the vessel from the side. The vessel when dilated permits the ready entrance of a No. 23 B. & S. gauge needle. However, the needle usually employed is a No. 26 B. & S. gauge, five-eighths of an inch in length. The needle is always introduced well into the lumen of the vein. If entrance into the vessel is direct, subsequent hemorrhage may be controlled readily by pinching it with a small forceps.

Rabbit.—For intravenous injection of the rabbit the following method may be employed:

1. The posterior auricular vein along the outer margin of the ear is better adapted than a median vein for this purpose.

2. If a number of injections are to be made commence as near the tip of the ear as possible, as the vein may become occluded with thrombi, and subsequent inoculations may then be given nearer and nearer the root of the ear.

FIG. 5.—INTRAVENOUS INJECTION OF A RABBIT.

3. The animal should be held firmly, as the slightest movement may result in piercing the vein through and through and requiring reinsertion of the needle. This is accomplished satisfactorily by placing the rabbit upon the edge of the table and holding it firmly there by grasping the neck and front quarters, the assistant at the same time compressing the root of the ear with the thumb and forefinger.

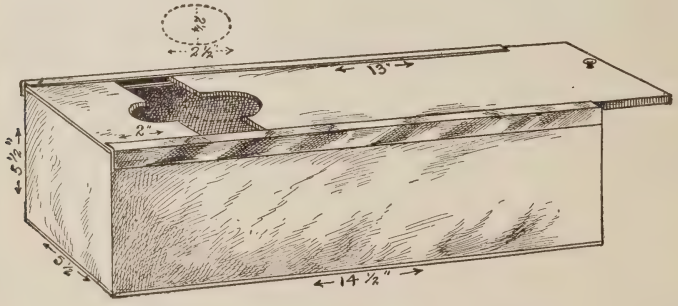

FIG. 6.—WOODEN BOX FOR HOLDING RABBITS FOR INTRAVENOUS INJECTION.

4. If the hair is long, clip it.

5. The ear is struck gently with the fingers and washed with alcohol and xylol; the friction will render the vein more conspicuous.

6. The ear is grasped at its tip and stretched toward the operator, or the vein may be steadied by rolling the ear gently over the left index-finger and holding it between the finger and thumb.

7. The inoculum should be free from solid particles and all the air excluded from the syringe. As a general rule, the amount injected should be as small

as possible, and the temperature of the inoculum be near that of the body. If the syringe is filled shortly after sterilization, when it has cooled enough to be comfortably hot to the touch, the heat will warm the injection fluid and not be hot enough to cause coagulation.

8. Hold the syringe as one would hold a pen, and thrust the point of the needle through the skin and the wall of the vein until it enters the lumen of the vein (Fig. 5).

The wooden box shown in Fig. 6 is very convenient for holding rabbits for intravenous injection or for bleeding from the ears.

9. Direct the assistant to release the pressure at the root of the ear, and slowly inject the inoculum. If the fluid is being forced into the subcutaneous tissue, which will be evident at once by the swelling which occurs, the injection must cease and another attempt be made.

10. The needle is quickly withdrawn, a small piece of cotton moistened with alcohol placed upon the puncture wound, and firm compression applied. Wash the ear thoroughly with alcohol and water to remove xylol, otherwise a low-grade inflammation will follow, which will render subsequent injections more difficult.

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PART II

CHEMOTHERAPY OF BACTERIAL AND MYCOTIC DISEASES

CHAPTER III

PRINCIPLES OF THE CHEMOTHERAPY OF BACTERIAL DISEASES

While the foundations of our knowledge of the chemotherapy of disease have been mainly based upon studies in protozoan infections and especially trypanosomiasis and spirochetosis, and while great achievements have been realized in this new therapy and especially in the treatment of syphilis and frambesia tropica, yet the bacterial diseases offer an equally important field and not a little work has already been done with encouraging results. The conquest of tuberculosis, pneumonia, typhoid fever, streptococcus infections and numerous other systemic and localized bacterial infections by specific chemical disinfection of the tissues constitutes a field of endeavor worthy of the unremitting toil and interests of those engaged in chemotherapeutic investigation. Theoretically, at least, one may expect even greater success in the chemotherapy of bacterial than protozoan and metazoan diseases, since these simpler organisms are even less closely related to mammalian organisms than the latter, but experience has shown that actually this is not the case, probably because, in part at least, of the simpler nature and life processes of the bacteria which are therefore more difficult to cripple and destroy than the protozoa possessing more numerous and important life processes presenting more vital points and greater opportunities for chemical attack and destruction.

Historical.—When one realizes that attempts to disinfect a wound or cure a localized infection of the skin or a mucous membrane by the application of chemical bactericidal substances constitutes a form of chemotherapy, it is apparent that the chemical disinfection of bacterial infections has been practiced empirically for many years and indeed, long before the discovery of bacteria and the specific nature of bactericides. The frequency with which wine was employed, either alone or in combination with other substances for the irrigation of wounds in the Middle Ages is an example, since the alcohol doubtless exerted slight bactericidal effects; even the hot iron applied to wounds for hemostasis in the times antedating the introduction of ligatures may have had the extra but unsuspected virtue of disinfection and especially when the resulting sterile eschar and exudate was protected to some extent against secondary infection by the frequent application of the wine and balsamic mixtures possessing some slight degree of antiseptic activity.

Hand in hand with the discovery of pathogenic bacteria went the discovery of the bactericidal activity of phenol, mercuric chlorid and a host of other chemical agents. Naturally most of these early investigations were conducted by test-tube or *in vitro* methods employing pure cultures and when the high bactericidal activity of phenol, mercuric chlorid and other substances was discovered, great hopes were entertained and expressed by Koch and other pioneers regarding the development of methods for the successful disinfection of local and systemic diseases of the body by the use of chemical agents. But it was soon found that these substances were so toxic or organotropic for the body cells or so rapidly altered by the blood and other tissues that sufficient could not be administered for the specific disinfection of the body in systemic diseases, although some success and encour-

agement attended the disinfection of wounds and various localized infections. But in surgical practice aseptic technic soon commanded greater attention than antiseptic technic, and because of the local irritation and absorption of toxic amounts of phenol, mercurials, and other germicides applied to infected tissues, the chemical disinfection of wounds fell into disuse until revived with much success during the recent World War by Carrel and Dakin employing antiseptics of the chlorin group.

Probably the first serious attempt to develop the chemotherapy of a generalized or systemic bacterial infection was made by Morgenroth and Levy in 1911, working with a derivative of quinin called ethylhydrocuprein (optochin) and experimental pneumococcus infections of mice. This work yielded encouraging results and led to the trial of optochin in the treatment of lobar pneumonia by Moore and others. The results, however, have not been satisfactory because the curative dose approximates the toxic dose too closely and because of the influences exerted by the pathological changes in pneumonia about to be discussed, but nevertheless the synthesis of optochin gave great impetus to investigation in the chemotherapy of bacterial infections and was soon followed by that of Browning and his colleagues in England with studies of the acridin dyes and in this country by Churchman, with gentian-violet and other triphenylmethane dyes. Both of these investigators revived interest in the *in vitro* methods for studying the specific parasitropism of dyestuffs, and their work, especially that of Churchman, has resulted in the development of useful and encouraging methods for the treatment of local and systemic infections caused by the pyogenic organisms.

Morgenroth has consistently devoted his efforts to the development of what is designated as "chemotherapeutic antisepsis" and his work, already fruitful in the production of new acridin derivatives, developed by studies employing experimentally produced local and systemic streptococcus infections of mice, along with that of Carrel and Dakin, Churchman, Young, White and their associates, the latter producing mercurochrome for the treatment of local and systemic bacterial infections, has greatly advanced our knowledge of the chemotherapy of bacterial infections and produced drugs of practical value in the treatment of several. To these achievements I may add the efforts of Schamberg, Raiziss and myself with the synthesis of new mercurial compounds and especially of mercurophen for the treatment of localized infections.

Localized versus Generalized Bacterial Infections, in Relation to Chemotherapy.—It is readily apparent that the principles and mechanism of the chemotherapy of localized bacterial infections by the direct application of disinfectant substances are quite different from those concerned in generalized or systemic infections requiring the injection of the disinfectant into the blood or its absorption from the gastro-intestinal canal, muscles or subcutaneous tissues. From the standpoint of chemotherapy, a localized infection may be defined as one confined to a certain organ or tissue and reachable by direct or topical application of a chemical disinfectant. It may be a localized infection in the strict sense of being a tissue or organ directly or locally infected by an organism, as for example, the urethra by the gonococcus, but in relation to chemotherapy, it may also include a tissue or organ infected by way of the blood stream but yet treatable by the local application of a medicament to the infected parts; for example, a joint may be infected by a streptococcus conveyed in the blood from the apical abscess of a tooth but may be amenable to treatment by the local injection of a chemical disinfectant. Infections of the skin, the accessory sinuses of the upper respiratory tract, gums, conjunctivae, urethra, bladder, etc., are usually localized infections in the strict sense, but pleuritis, meningitis and infections of other serous cavities may be also classified as localized infections from the chemotherapeutic standpoint since they may be treated by direct local or topical application or injection of disinfectant substances even though the organisms are brought to the parts by systemic channels. On the other hand, certain infections of other organs as those of the iris, gall-bladder, perirenal tissues, etc., may be strictly localized but reachable therapeutically only by way of injection of medicaments directly into the blood or indirectly by absorption from the subcutaneous tissues, muscles or gastro-intestinal tract.

In other words, generalized or systemic infections are those in which organisms occur constantly or intermittently in the blood or lymph streams

with one or several localizations in the fixed tissues; usually they are treatable only by way of the lymph or blood streams by direct injection of medicaments into the blood or absorption from the subcutaneous tissues, muscles or gastro-intestinal tract. Localized infections are those in which the organisms occur in the infected parts as the result of a direct infection or by localization of organisms from the blood; according to accessibility of the part infected they may be treated by the topical application or systemic administration of drugs.

Principles of the Chemotherapy of Localized Bacterial Infections by Topical or Local Medication.—As previously stated, it is but natural that the treatment of wounds and superficial infections of the skin and mucous membranes by topical or local applications of chemical disinfectants should constitute the earliest empirical attempts in chemotherapy, even before the discovery of bacteria and their relation to infection. But with discoveries in bacteriology and of germicidal substances, the first practical application of knowledge regarding the latter was in the treatment of localized infections by topical applications. Upon first thought it does not appear that Lister's method of antisepsis by the carbolic acid spray was an application of chemotherapy, but in a strict sense this was true, since it constituted an attempt to selectively destroy organisms with a chemical agent without injury to the cells of the host. Owing, however, to the fact that the patient was sometimes poisoned locally or systematically by the phenol employed and since it was found that aseptic methods yielded better results than antiseptic methods in surgical operations, the method was discontinued.

But upon the discovery of the high bactericidal activity of the salts of mercury and other heavy metals as well as of alcohol and various other substances, interest in the treatment of infections of wounds and of local accessible tissues in general by the application of solutions of these substances was greatly renewed. It was soon observed, however, that the bactericidal activity of substances in the presence of blood and the tissue juices was much less than in the test-tube; likewise that the bactericides were unable to penetrate leukocytes and fixed tissue cells sufficiently well to reach organisms and that the agent may produce sufficient local irritation to preclude its use or result in the absorption of sufficient amounts to produce general poisoning. As a means for the treatment of infected wounds the topical application of bactericides was gradually discontinued, but never entirely abandoned, and during the World War was successfully revived by Carrel, Dakin and others. In other words, the practice involved has always been correct in principle; there was simply a failure to properly understand the technical and other factors guiding the selection of disinfecting agent and the manner of its application. Every time the surgeon disinfects the skin with iodin, picric acid or other chemical agent, he is practicing chemotherapy in a general way, since he seeks to reach and destroy organisms with a chemical agent employed in such strength as accomplishes this purpose without apparent injury to the patient. Every time an infected wound is irrigated with Dakin's or other germicidal solution by Carrel's or some other method, chemotherapy is being practiced in practically the same sense as the application of solutions of gentian-violet for the selective destruction of diphtheria and other Gram-positive organisms in wounds, as described by Churchman.

And the treatment of erysipelas and various other infections of the skin by applications of solutions of iodin, boric acid, mercurochrome and other agents, the application of solutions of boric acid, the silver salts, etc., to the conjunctival, the nasal mucosa and accessory sinuses, the urethra, etc., are likewise examples of chemotherapy in the empirical sense, long practiced

and generally useful since all represent attempts to destroy bacteria by the local or topical application of chemical agents employed in solutions of such strength as are bactericidal without demonstrable organotropic or toxic effects.

But what are the guiding principles of these very practical and important applications of the chemotherapy of localized infections? Does success in the sterilization of a wound, the skin or a mucous membrane depend entirely upon or necessarily bear an important relation to, the actual bactericidal activity of the chemical agent employed? Despite the fact of numerous mistakes and disappointments in the past on this score, the impression prevails that this is a consideration of first importance, but a close examination of the facts shows that it is not necessarily so and that other factors may be of equal or even greater importance. For example, a saturated solution of boric acid in water does not rank at all high in bactericidal activity, even under the best of conditions in the test-tube, and yet it constitutes one of the best and most successful topical applications for pyogenic infections of the skin and mucous membranes with which I am familiar, probably largely because of its osmotic absorption. The antiseptics of the chlorin group may be cited as additional chemical agents relatively feeble in bactericidal activity, but yet wonderfully efficient for the local disinfection of wounds when properly prepared and applied. And the list may be extended to include some of the organic silver salts, some of the acridin and triphenylmethane dyes, some of the essential oils, etc., possessing relatively feeble bactericidal activity as compared with the inorganic salts of mercury, but yet efficient disinfectors by topical application. From the standpoint of chemotherapy it would appear sufficient for a chemical agent to possess a moderate degree of bactericidal activity in the presence of blood, pus and other albuminous substances when applied in concentrations that are without local or systemic toxic effects for the cells of the host; in other words, mere superior bactericidal activity is not alone a criterion of success in the treatment of localized bacterial infections.

In addition, the disinfectant should possess the property of penetrating cells and tissues to reach organisms in the lymph spaces, lymphatic and vascular channels, as well as those protected by location in the protoplasm of leukocytes and other cells. If, in addition, the disinfectant has a dissolving effect upon dead cells and other organic matter (pus), an advantage is gained as shown, for example, in the effects of neutral sodium hypochlorite solution in the disinfection of infected wounds bathed in pus and necrotic tissues. Even the property of mechanical cleansing by floating pus from the depths to the surface is a matter of importance and the toxic qualities of the disinfectant for individual cells and especially phagocytes, as well as for complement and bactericidans in the blood and tissue fluids, are matters of primary interest and importance, as well as the question of local irritation with the production of pain and systemic poisoning from absorption.

Essential Properties of a Chemical Agent for the Chemotherapy of Localized Bacterial Infections by Topical Application.—If such are the main principles of the chemotherapy of localized infections of wounds, the skin, serous cavities (pleural, peritoneal, subarachanoidal, synovial, etc.) and such accessible mucous membranes as those of the urethra, bladder, upper respiratory tract, etc., it may be well to briefly summarize here our ideas of the essential properties to be possessed in whole or part by agents offered for the chemical disinfection of these tissues by direct or topical application:

1. *They should possess some degree of bactericidal and bacteriostatic activity* in the test tube and tissues, and especially in the presence of serum, blood, pus, etc. As previously

stated, these effects need not be extremely high and they may be selective, as for example, gentian-violet and other dyes of the triphenylmethane series for Gram-positive bacteria like staphylococci, streptococci, pneumococci, diphtheria bacilli, etc. While an extremely high bactericidal or bacteriostatic activity *in vitro* is not essential yet it is desirable, as such a compound is more apt to retain effective degrees of bacteriostatic activity *in vivo*. Furthermore, if the chemical agent possesses a high degree of selective bacteriostatic power, it may fail in the treatment of a mixed infection. For example, Bashford and his colleagues (1) found that in infected wounds treated with Dakin's solution, it was the cocci which disappeared last, while in wounds treated with flavin the commonest type of organism to outlive others on the wound surface was a Gram-negative bacillus. The same kind of result was obtained in Churchman's (2) efforts to sterilize amputation stumps with gentian-violet. It was comparatively simple to get rid of the Gram-positive *Bacillus diphtheriae*, but very difficult to abolish the Gram negative *Bacillus coli*.

2. *In addition they should possess the property of destroying toxins and especially the toxins of the tetanus and other anerobic bacilli of infected wounds;* in this connection it may be stated that the oxidizing disinfectants, as those of the chlorin group, are believed to have this property.

3. *They should not be precipitated or their bactericidal properties neutralized by the proteins or salts of the polybasic acids of the body fluids and tissues* with which they come in contact, as by pus and necrotic tissues in wounds or serous cavities, by urine in the pelvis of the kidney or bladder, etc.

4. *They should possess the property of penetrating cells and tissues and especially phagocytic leukocytes and other cells in which living bacteria may be lurking.* This is one of the most important principles of all and especially in the treatment of chronic infections of mucous membranes in which the bacteria are almost sure to have taken up a position of grest strategic strength in the depths of glands and cells.

5. *They should be free of local organotropic or toxic effects for the fixed tissues*, that is, free or almost so, of irritating and inflammatory properties; on the contrary they should be of a soothing or bland nature and preferably possess some degree of analgesic effect.

6. *They should not be excessively toxic for the body as a whole in case considerable amounts are absorbed.* This is especially important in the irrigation of wounds and much less so in infections of the skin and mucous membranes, the whole subject bearing a direct relation to the question of absorption.

7. *They should exert a chemotactic effect*, that is, attract leukocytes about the focus of infection and thereby aid in the walling off of the involved area and possibly aid phagocytosis and the elaboration of cellular proteolytic and lipolytic enzymes. At least, they should not repel or destroy leukocytes and thereby favor the extension of the infection.

8. *They should not destroy or paralyze the immunological substances like complement, bacteriolysins and opsonins (bacteriotropins)* concerned in the destruction of organisms.

9. *They should be rapid in bacteriostatic and bactericidal activity.* This is especially important in the treatment of such infected surfaces as the urethra, conjunctiva, etc., where the contact of disinfectant is extremely brief. Indeed, this largely accounts for failure in practice and it is commonly observed that clinical results are very much better when the disinfectant is retained for some time, as, for example, in the case of instillations into the urinary bladder or when it is frequently applied, as in the Carrel method of irrigation of wounds. But whenever the conditions are such that prolonged contact is not possible, rapid penetration and rapid bactericidal effects become of increasing practical importance.

10. *They should possess some degree of dynamogenic activity*, that is, the property of stimulating the fixed tissues to proliferative activity as a further aid in the walling off of lesions followed by cutting off oxygen and blood supply to the organisms and thereby allowing their own acid and waste products to check their growth and eventually destroy them.

Methods of Topical Treatment of Localized Infections in Relation to Chemotherapy.—But a compound possessing all or the majority of these essential and desirable properties may yet fail to prove useful in the treatment of localized bacterial infections because it may not be possible to apply it correctly. Indeed, I believe that the essential reason for the skepticism of surgeons regarding the practical value of topical applications of chemical disinfectants in the treatment of infected wounds, serous cavities, sinuses, etc., is due largely to the difficulties or impossibility of correct application. Merely to apply the disinfectant on gauze is usually insufficient as this may only protect against secondary infection without appreciably influencing the existing infection. As so clearly shown by Carrel and his colleagues with

the chlorin group of antiseptics in the treatment of infected wounds, it is essential to bring the disinfectant solution into frequent and intimate contact with the infected tissues along with the surgical removal of necrotic tissues when possible and advisable and always the establishment of the best possible drainage. Furthermore, these ends should be attained with the least meddling and disturbance in order to permit granulation and healing to proceed; for these reasons Carrel has contributed his valuable method of irrigation with rubber tubes laid in the wound as a substitute for frequent syringings and packings.

The method of application, therefore, is of considerable importance; indeed, I believe it decidedly outranks in importance the choice of antiseptic solution itself provided the latter approaches in blandness simple physiological saline or Locke's solutions with a reasonable degree of bactericidal activity, and the essential principles involved may be summarized somewhat as follows:

1. Free drainage is of the utmost importance and should be provided for by surgical means if necessary. This will facilitate better access of the disinfectant to the infected tissues, reduce dilution of the disinfectant to a minimum, reduce the degree of absorption of toxic substances from bacteria and necrotic exudates and tissues and minimize the danger of spreading the infection by over-distention of the tissues when a cavity is present.

2. In infected wounds, bits of necrotic tissue should be removed surgically, if possible, without too much danger of spreading infection, in order to remove or prevent the formation of pockets constituting good breeding places for bacteria, particularly the anaërobes, and which may be a constant source of re-infection of the tissues.

3. The disinfectant solution should be frequently applied. In the Carrel method employing a chlorin antiseptic this may be as often as every two hours. To apply the disinfectant once a day at the time of dressing the parts is usually insufficient and merely a cleansing process, because of the very brief contact between disinfectant and the tissues. Even though the lesion may not demand a complete set of Carrel tubes, the proper placement of a single tube of the right kind with the frequent injection of disinfectant with a syringe may serve the purpose without disturbing the dressings. It is largely because frequent applications are not usually possible in the treatment of chronic infections of the nasal accessory sinuses, that their treatment is so generally unsatisfactory; on the other hand, in acute and chronic conjunctivitis the method of frequent applications is usually practiced and for this reason excellent results are obtained by the instillation of medicaments possessing but feeble bactericidal activity but serving a very useful purpose by merely cleansing the infected tissues with frequent removal of pus, bacteria and toxins.

The correct principles, therefore, are free drainage and frequent applications of a disinfectant solution possessing the essential properties described above. Under these conditions bacteria and their toxins, as well as the toxic substances of protein digestion of the necrotic tissues and exudates, are being constantly removed and their absorption reduced to a minimum; in addition enormous numbers of bacteria may be killed, or at least their proliferation prevented, if the disinfectant fails as a bactericide but functions as an antiseptic or bacteriostatic agent. Such management permits phagocytosis to proceed by removing the aggressin-like bacterial toxins which tend to prevent this important curative process; the extension of bacteria along the lymphatics is discouraged; the danger of development of bacteremia (septicemia) is greatly minimized and the degree of sapremia from absorption of toxic exudates is reduced.

4. Finally, the disinfectant should be introduced with practically no pressure in order to avoid over distention of the tissues, which may disseminate the infection. Gentleness and the minimum of handling are of primary importance.

Varieties of Localized Infections in Relation to Chemotherapy.—But, of course, not all localized bacterial infections demand disinfectant treatment. Judgment must be exercised according to the kind of organism present and the duration and extent of the lesion. Ordinary acute staphylococcus abscesses, for example, usually do best with simple incision for adequate drainage and much handling, squeezing and syringing are to be avoided. As a general rule, when the incisions of a localized lesion are only large enough for drainage, no attempt should be made to disinfect because

of the danger of blocking drainage by additional tubes, but in large lesions, as infected wounds, widely incised staphylococcus and streptococcus abscesses, streptococcus infections of the uterus, etc., where provision may be made for both drainage and irrigation, I believe that disinfection at short intervals is indicated and especially in streptococcus infections.

It is true that disinfectant solutions may not reach or influence those bacteria already deep situated in the tissues and especially in the lymphatics, but the proper kind of disinfectant solution along with drainage may tend to reverse osmosis and thereby reduce the degree of absorption of bacteria and toxins and at least greatly reduce their production which may be a factor of no little value in streptococcus and pneumococcus infections. Furthermore, some localized bacterial lesions cannot be subjected to frequent disinfection because of anatomical difficulties. For example, in pneumococcus and streptococcus cerebrospinal meningitis adequate drainage and disinfection are not ordinarily provided for and I am convinced that daily or twice daily spinal drainage with the injection of a serum or chemical disinfectant in the lumbar region are insufficient. It is imperative for surgeons to perfect a method for frequent drainage if anything worth while is to be accomplished in these dreaded and highly fatal infections; if, in addition, ways and means are provided for cleansing and disinfecting the subarachanoid space at frequent intervals, the chances of success will be much greater. But the problem of the disinfectant is practically solved; it awaits the development of better surgical procedures for its introduction and adequate drainage. The ear and accessory sinuses of the nose likewise present difficulties and for this reason oto-rhinologists have not found disinfectant solutions of particular value in treatment; but I believe this is largely due to the fact that the disinfectant cannot be kept in contact with the infected tissues for sufficient periods of time and indeed, may not reach some deeper parts at all, although it is possible that gaseous disinfectants of the chlorin type may be employed with greater success and especially if means are provided for the patient to apply these at frequent intervals.

But in the treatment of chronic empyema, chronic suppurative arthritis, chronic suppurating sinuses, osteomylitis, endometritis, etc., the possible advantages from well directed attempts at disinfection at short intervals should not be overlooked or minimized and I believe these localized infections constitute a proper field for coöperation between surgeon and chemotherapeutist; for this reason I shall be bold enough to offer advice in regards to local disinfection when taking up for consideration the practical application of chemotherapy in the treatment of localized and systemic bacterial infection in Chapter VIII.

Chemotherapy of Localized Bacterial Infections by the Systemic Administration of Medicaments.—Is it possible to disinfect a strictly localized bacterial infection by the administration of a compound by intravenous or subcutaneous injection or oral administration? This is a very broad and important question involving the present state of the chemotherapy of bacterial diseases in general since tuberculosis, bacterial endocarditis, pneumonia, puerperal sepsis, etc., may be localized infections in just the same sense as an abscess at the root of a tooth, a sinusitis, a staphylococcus or streptococcus cellulitis and lymph-adenitis, etc.; it involves primarily the question of penetration of chemical agents from the blood into the tissues of bacterial infection, which will be shortly discussed with detail, but here it may be stated that a general answer cannot be given, since it would appear that the matter of penetration varies according to the infection and the kind of compound administered. For example, it is doubtful if we have available

at present a chemotherapeutic agent capable of penetrating tubercles although some of the dyes may do so; on the other hand, clinical reports indicate that gentian-violet and mercurochrome are apparently able to penetrate localized infections caused by *B. coli*, straphylococci and streptococci although Hirschfelder, Malmgren and Creavy[3] have recently reported that while mercurochrome and acriflavin may penetrate gradually from the blood into the edema fluid produced by paraphenylendiamin, this does not occur with doses proportional to those employed clinically and that neither these compounds nor gentian-violet in such amounts rendered the edema fluid bactericidal or bacteriostatic. I have repeatedly observed that the intravenous injection of mercurochrome, gentian-violet and acriflavin failed to sterilize the pus of abscesses at the roots of teeth showing hemolytic and non-hemolytic streptococci in cultures; also that pneumococci in the pus of infections of the nasal accessory sinuses were apparently untouched, but mercurochrome in my experience has apparently penetrated acute staphylococcus and streptococcus lesions of the subcutaneous tissues, lymph-glands and endometrium with curative effects. As previously stated, I shall shortly discuss this subject with more detail, but here it may be stated that in severe acute localized bacterial infections it may be possible to re-enforce disinfection by local or topical application of disinfecting agents by the systemic administration of a medicament and especially by the administration of mercurochrome.

Related to this subject of specific chemotherapeusis of local bacterial infections by the topical or local application or systemic administration of a disinfectant agent, is the possibility of treating infections of the organs of the urinary, biliary, gastro-intestinal, respiratory and cutaneous systems by the administration of compounds largely eliminated in these tracts. Hexamethylamin, for example, apparently owes a part of its beneficial effects in the treatment of infections of the genito-urinary organs to the elimination of small fractions of formaldehyd liberated in acid urine, although it is doubtful if sufficient formaldehyd is liberated in other tissues as in the bile, milk, saliva, cerebrospinal fluid, etc., to produce beneficial results and hexamethylamin itself is practically free of bactericidal effects in the amounts ordinarily eliminated unchanged. Recently it has been shown that mercurochrome and hexy-resorcinol administered by ingestion or intravenous injection may be eliminated in the urine in sufficient amounts to render it bactericidal and the same may be true to a lesser extent of other mercurial compounds. It is well known that arsenic is largely eliminated in the bile, but unfortunately the bactericidal activities are so feeble that curative effects in bacterial infections of the liver, gall-bladder and ducts are not apparent. It is not improbable, however, that mercurial compounds eliminated in the bile may reach bacteriostatic concentrations and even reduce to some extent the bacterial flora of the small intestines. The beneficial effects of creosote, guaiacol and their derivatives in bronchitis are apparently due in part to their elimination in the bronchial secretions, but I do not know of any drug eliminated by the skin exerting curative activity in bacterial infections of this organ.

But the principle is well established and worthy of much more attention than has been heretofore given this subject. This is especially true of bacterial infections of the genito-urinary organs and biliary passages. It is probable, however, that compounds eliminated in the urine and bile may be so profoundly altered that bactericidal effects are lost; this is apparently true of the organic trivalent arsenicals like arsphenamin and neoarsphenamin, which are largely eliminated in the bile as inert pentavalent compounds and

half- or pseudo-complex compounds of mercury may become dissociated and combine with proteins and thereby loose greatly in bactericidal effects, etc. Unfortunately, the converse of this is apparently of infrequent occurrence; that is, relatively inert compounds do not acquire bactericidal effects during elimination except in the case of hexamethylamin when traces of formaldehyd are liberated in an acid medium. But it may be that similar effects are more common than we now suppose and thereby explain such beneficial results as those observed, for example, with creosote, guaiacol and their derivatives in bronchial infections, the salicylates in acute rheumatic fever, etc., which possess but feeble bactericidal effects *in vitro*.

In a broad and general manner, it may be stated that the secretions of organs are much less likely to eliminate drugs to a sufficient extent to secure curative effects in bacterial infections than the excretions. For example, we do not possess at present a bactericidal drug capable of passing the choroid plexus in meningitis; hexamethylamin may pass, but bactericidal amounts of formaldehyd are not liberated. But in recent experiments I have found that traces of ethylhydrocuprein administered by intravenous injection and even by ingestion to dogs and rabbits, may occur in traces in the cerebrospinal fluid in pneumococcus meningitis and I have likewise detected mercury in the fluid in experimental streptococcus meningitis following the intravenous injection of mercurochrome. In other words, while drugs may not find their way into the subarachnoid space under normal conditions, yet such may occur as the result of acute inflammatory changes of the meninges and choroid plexus.

Examples of these kinds could be further multiplied, but I hope my purpose is fulfilled by those given, which is to draw more attention to the possibilities of influencing infections through the action of disinfectants eliminated in the excretions and secretions of the body modified by disease. In this connection it is hardly necessary to emphasize that studies upon elimination by *normal* organs are apt to be insufficient; for example, when we approach the matter of elimination of disinfectants in the cerebrospinal fluid for the treatment of meningitis, we should experimentally produce a meningitis in the animal and the same is true in studies of elimination by bile. There is, in other words, likely to be considerable difference between elimination by a normal organ and elimination by a diseased organ and our experimental conditions should approximate those of the disease we seek to influence or cure.

Principles of the Chemotherapy of Bacterial Infections by Systemic Medication.—By systemic medication I mean the administration of medicinal agents by ingestion or injection (subcutaneous, intramuscular, intravenous, etc.) and their subsequent distribution by the blood. This introduces new problems concerning the fate of the compound before it has had an opportunity for attacking the organisms and the opportunities and chances for effective disinfection are greatly reduced in comparison with disinfection by direct application.

In the first place, the compound is immediately diluted to a high degree in the blood and other body fluids and this alone may suffice for reducing it below a bactericidal concentration. Furthermore, elimination is likely to begin almost immediately and this, along with the rapid absorption or removal of the compound from the blood by physical or chemical union with the fixed tissue cells or its rapid conversion into an inert compound, may result in a still greater reduction in bactericidal activity and effectiveness. It is true that none of these unfavorable influences may be sufficiently prompt or powerful to prevent the compound from disinfecting the blood, but they are

apt to be very real obstacles in the path of a disinfectant between the site of its administration and the organisms in the fixed tissues protected by edematous fluid, necrotic cells and vascular occlusions. For these reasons chemotherapy may be much more effective in the disinfection of the blood and in keeping the blood in a sterile condition than in the disinfection of a bacterial infection localized in some tissue or organ and, indeed, my clinical and laboratory experience indicates that such may be true in streptococcus and pneumococcus infections.

On the other hand, a compound may undergo an increase rather than a decrease of parasitropic activity in the blood. It would appear that this occurs in the case of arsphenamin and other trivalent arsenicals by the formation of the trivalent "oxids" of which arsenoxid is the type, and apparently some analogous change must occur in the case of the salicylates to account for their curative effects in acute rheumatic fever. Indeed, I am inclined to believe that progress in chemotherapy has been hampered somewhat by placing too much emphasis upon bactericidal effects *in vitro* and discarding new compounds on the basis of low bactericidal activity without giving them a trial *in vivo* for the possible chance of their being more parasitropic under such conditions than we may naturally expect. Mercurochrome for example, would never greatly impress one as a compound of promise in the treatment of a bacterial infection because of its relatively feeble bactericidal activity *in vitro* in comparison with gentian-violet, but yet, *in vivo*, mercurochrome has proved more effective in my experience than the latter largely because gentian-violet is rapidly reduced to an inert compound while mercurochrome is more stable even though it does not appear to be converted into a new or more active substance.

But the disadvantages of rapid dilution, rapid elimination and rapid fixation by the tissue cells are to be reckoned with in any event and demand that they be taken into consideration in our dosage and method of administration in order to maintain an effective concentration of the drug in the blood for suitable periods of time.

In addition to these factors involving the metabolization of the compound, it must be of sufficiently low toxicity or organotropism to permit of administration of adequate amounts without undue risks of temporary or permanent damage to physiological important cells and organs and it must possess the property of penetrating new bacterial tissues and exudates in an active state for the purpose of effecting their disinfection. The latter is indeed a consideration of first importance in the chemotherapy of all bacterial infections other than of bacteremias or septicemias. It would appear to be the main stumbling block in the development of an effective chemotherapy of tuberculosis, pneumonia, actinomycosis and such infections characterized by profound pathological tissue changes. The laws governing this important subject of capillary permeability and the penetration of lesions by chemical agents have not been worked out although it is a problem of first rate importance. It may be that the determining factor is a matter of the chemistry of the organisms as influencing affinity for bactericidal compounds, but it is more likely to be one of physics involving the simple laws of diffusion of crystalloids and colloids as well as molecular size or magnitude. The pathology of a disease may, therefore, profoundly influence its chemotherapy and largely by its modifying influence upon capillary permeability and tissue penetrability, as I shall shortly discuss with more detail.

Essential Properties of a Chemical Agent for the Chemotherapy of Bacterial Diseases by Systemic Administration.—With these considerations in mind we may now briefly summarize the essential properties of a chemical

agent in relation to the chemotherapy of bacterial diseases, when administered by ingestion or injection and presumably distributed by the blood and other body fluids:

1. It must be of sufficiently low toxicity for the body cells to permit of the safe administration of adequate amounts without delayed toxic effects or the production of too violent immediate reactions by incompatibility with the blood.

2. It must be sufficiently toxic for the bacteria to exert bactericidal and bacteriostatic effects. For this purpose it must be either sufficiently high in bactericidal activity to be effective in the blood despite high dilution or be a compound convertible in the blood and tissues into a new compound of higher parasitropic activity.

3. It must not enter too rapidly into physical or chemical union with the constituents of the blood or fixed tissues with the production of inert compounds.

4. From the standpoint of the treatment of tuberculosis and other bacterial infections characterized by the production of pathological exudates and tissues, it must possess the property of penetration sufficient for disinfective purposes by conforming to the physical laws of diffusion or by possessing a chemical affinity for the proteins or other constituents of the organisms according to Ehrlich's conception of parasitropic activity. This, however, is not required for the disinfection of the blood and lymph although an affinity between compound and organism must exist.

5. The compound should not depress immunological processes and especially phagocytosis. On the other hand, it should produce leucocytosis and enhance phagocytosis in both the blood and fixed tissues.

6. In addition to these desirable qualities, the curative activity of a chemotherapeutic agent or drug may be enhanced if it possess some degree of dynamogenic and chemotactic activity, which is the property of exciting hyperemia about lesions with serous and cellular exudation and promoting the proliferation of connective tissue which tends to wall of chronic lesions and gradually aid in their disinfection by cutting off oxygen and food supply and allowing the acids and waste products of bacteria and the products of caseation and liquifying necrosis to check and eventually destroy the organisms.

Pathology of Bacterial Diseases in Relation to Chemotherapy.—As would be expected better results are observed in the chemotherapy of acute than of chronic bacterial infections. Probably best results of all are secured in the disinfection of the blood in cases of bacteremia (septicemia). This is true because we have a few drugs like ethylhydrocuprein hydrochlorid, mercurochrome and gentian-violet which may be given in sufficient amounts to temporarily raise the bactericidal titer of the blood for effective sterilization although it may become re-infected a few hours later by organisms from localized lesions escaping disinfection.

But organisms in the fixed tissues can only be reached and destroyed when sufficient amounts of the compound in an active bactericidal state leaves the blood and permeates the lesion. This in turn involves the questions of rapidity of elimination, production of inert combination compounds, the physical laws of diffusion, capillary permeability, etc., discussed above; it also involves the degree and kind of pathological tissue changes which may be present and constituting the theme of the present discussion.

In acute bacterial infections the hyperemia is undoubtedly a favoring factor for bringing the bactericide to the sites of infection and during the processes of acute cellular and serous exudation a part of it may be swept along into the inflammatory exudates even though the compound itself does not possess those physical characteristics which facilitates its passage through the walls of normal vessels. Permeability is of fundamental importance, but in acute inflammatory lesions it may not be as important as in chronic lesions because of the opportunities of the compound being carried along mechanically in the former into the infected tissues by the processes just mentioned. We may expect, therefore, better results in acute than in chronic infections. But even when compounds like mercurochrome, acriflavin, gentian-violet, optochin, etc., leave the capillaries, their diffusion may be hindered by the physical characteristics of the edematous fluid and

pus as well as by the leukocytes and other cells containing many of the organisms. The problems, therefore, are many and difficult and particularly good results may not be obtained until we learn more about the laws governing permeability and diffusion under these circumstances and until we succeed in evolving compounds which will accumulate in the lesions to reach bactericidal concentrations, which I believe occurs with mercury in the treatment of syphilis and explains its peculiar efficacy in this disease.

In chronic bacterial diseases the lesions may be quite avascular, as in the case of tuberculosis; furthermore, many of the blood-vessels are apt to be sclerosed which may amount to occlusion of the smaller vessels. Serous and cellular exudation are reduced to a minimum and we have therefore a set of circumstances very unfavorable for chemotherapy and especially if our compounds possess but feeble permeability for capillaries at best and a low diffusibility in the products of caseation and liquifying necrosis.

Needless to state the pathological lesions produced by bacterial infections may also influence the organotropism or toxicity of compounds for the body cells. It is commonly observed that the tolerance of infected animals for a drug is appreciably less than in normal animals and I believe the same is true of human beings. The bacterial toxins and exudates are always likely to produce albuminous degeneration of the kidneys, heart and other organs which may proceed to more advanced lesions and interfere with elimination to such an extent as may increase the chances for toxic effects and especially in the case of chronic diseases. On the other hand, however, just the opposite may occur during the acute stages; for example, the patient with croupous pneumonia has, I believe, a higher tolerance for optochin and quinin compounds in general than normal human beings. This is analogous to the increased tolerance of syphilitics for mercury and of malarial infected individuals for quinin. In other words, the toxins of the disease or some other agencies may have a de-toxifying effect upon the drug administered.

Even in localized bacterial infections pathological tissue changes may profoundly influence the results of chemotherapy by topical or direct application. In acute lesions the organisms are more superficial and, therefore, of easier access than in chronic lesions; in the latter, the bacteria are apt to be in the depths of glands and even in the submucous tissues protected against destruction not only by this location but likewise by the production of inflammatory exudates and fibrous tissue. It is for these reasons, therefore, that compounds possessing penetrability in greatest degree like the stains and dyes, are most likely to be successful. Indeed, I believe we may go so far as to state that compounds lacking in this quality can never prove very useful in the treatment of chronic infections by direct application, as they come in contact and destroy only those organisms swept out to the surface by the secretions and excretions and many of which are already dead, without being able to penetrate and reach the real offenders deeply and safely lodged in the tissues.

Dosage and Route of Administration in Relation to Bacterial Chemotherapy.—Naturally enough, these considerations also bear an important relation to our methods of administering compounds in the chemotherapy of bacterial diseases and especially by systemic routes of administration. In the treatment of the septicemias, it would appear advisable to give large doses by intravenous injection in order to raise the bactericidal activity of the blood to a high level even if for but a short period of time, as one to three hours. Small, frequently repeated doses may fail to accomplish disinfection if elimination and metabolization prevent the compound from reaching a bactericidal concentration in the blood under these circumstances, although

this method of administration may greatly aid in keeping the blood sterile, as during croupous pneumonia, streptococcus cellulitis, anthrax, etc.

But how may we encourage the penetration of chemotherapeutic drugs into the fixed tissues infected with bacteria? Experiments and clinical experience indicate that this is likewise probably best accomplished by large single doses although it may be shown in the future that it can be better accomplished by smaller and more frequent doses and especially if compounds are discovered possessing a higher affinity for bacterial tissues than is now apparently the case with the drugs so far produced. In other words, I believe that the correct method of administration with the compounds now available is to hit hard and quickly over a short period of time in the treatment of the acute bacterial diseases and especially the septicemias. With mercurochrome this usually means a dose of about 0.005 gm. per kilo of weight or approximately 23 c.c. of a 1-per cent. solution for an adult weighing from 130 to 140 pounds; but instead of giving all of this in one dose, it may be possible to secure results by giving 10 c.c. daily for three or four days in succession and I have had a few cases in which this plan has apparently been efficacious although in general terms better results have followed the slow injection of 25 to 30 c.c. followed by the acute reaction of chill, fever and diarrhea. This would appear to be the best means for disinfecting the blood and for encouraging penetration of the fixed tissues and exudates. Exactly the same principle pertains to the treatment of the acute bacterial diseases with immune serums, since better results appear to follow large doses at short intervals by intravenous injection over a period of a few days than small doses over a long period.

But in chronic bacterial diseases in which the blood is sterile the chief purpose is to encourage penetration of the lesions with the medicament. At present we do not appear to have a compound at our disposal which possesses an affinity for the bacterial tissue and a tendency to accumulate there in a manner similar, for example, to the accumulative effects of small doses of mercury over a long period of time in the treatment of syphilis, unless it be the salicylates in acute rheumatic fever. Experiments and clinical experience indicate that best penetration is probably secured by bringing about the highest concentrations in the blood, even if but for a few hours at a time, so that we are compelled to adopt the practice of large doses in a short interval as preferable to small doses over a long interval. Unfortunately this practice is not without danger, since the amounts of optochin, mercurochrome, gentian-violet, acriflavin and other drugs employed at present in bacterial chemotherapy required to produce curative effects may be so near their toxic doses that repeated large doses at close intervals may be dangerous and requiring considerable clinical experience and judgment in the selection of drugs, their doses and route of administration.

Influence of Chemotherapy upon the Immunological Changes of Bacterial Diseases.—Until we succeed in producing far more satisfactory chemotherapeutic compounds for the treatment of bacterial diseases than available at present, recovery will continue to depend largely upon immunological processes aided in some diseases by biological therapy (vaccines, serums, blood transfusion, etc.). The question naturally arises whether or not it is possible for us to aid Nature in her immunological processes by the administration of drugs? In other words, may drugs to be classed in the domain of chemotherapy increase antibody production or aid and facilitate phagocytosis in bacterial diseases?

We hear and read a good deal about arsphenamin and its congeners increasing antibody production or aiding immunological processes in syphilis

and trypanosomiasis, but the evidence presented so far is not very convincing, as I shall discuss with more detail in Chapter XII. In bacterial diseases, however, acquired immunity is developed to a greater degree and plays a far more important part in recovery than in syphilis and anything that may be safely done to hasten or increase these beneficial changes is to be welcomed.

In the first place, the intravenous administration of mercurochrome, other heavy metals and some of the dyes, quinin compounds, etc., commonly employed at present in bacterial chemotherapy, may increase the leukocytes of the blood and especially the polymorphonuclear neutrophils which we regard as playing a very important part in natural and acquired resistance and immunity. The doses required, however, for this result are usually the maximum amounts by intravenous injection. Unquestionably it is reasonable to expect that these leukocytes will prove of aid in overcoming the infection and the clinical reactions following the intravenous injection of mercurochrome and other heavy metals, bears such a close resemblance to the so-called non-specific protein reactions produced by the intravenous injection of peptone, vaccines, etc., that one cannot escape the suspicion that a goodly part of the curative results may be due to the same mechanism as called into play by the administration of these protein agents.

But I have had so far no clear or conclusive evidence of the increased production of such humoral antibodies as the antitoxins, agglutinins, precipitins, opsonins and bacteriolysins during the course of experimental or natural bacterial infections, to be ascribed to the influence of such drugs as optochin, gentian-violet, acriflavin and mercurochrome. It may be that such compounds actually stimulate the production of antibodies, since the production of leukocytosis indicates that the bone marrow and the antibody-producing tissues in general may be stimulated, but it is difficult or impossible with present test tube methods to elicit and present the evidence. It is true that the administration of these compounds to normal animals may increase temporarily their resistance to bacterial infection in a manner analogous to the temporary resistance of mice and rats, treated with some of the dyes, to inoculation with pathogenic trypanosomes, but the resistance is of such short duration that one cannot be sure that the results are not due rather to a retention of some of the compounds in the blood and tissues than to an increase of immunological resistance.

However, we do have some evidence to indicate that optochin, at least, and possibly also gentian-violet and mercurochrome may aid phagocytosis *in vivo* by lowering the resistance of the organisms. This is especially true in the treatment of local infections by topical application, but in this connection one must remember the possibility of paralyzing the leucocytes and retarding phagocytosis by the use of too strong solutions.

It may be, therefore, that some of our chemotherapeutic compounds may actually aid in immunological resistance and recovery from experimental and natural bacterial diseases of human beings and the lower animals, not so much by the actual stimulation of production of humoral antibodies, as by lowering the resistance of bacteria to phagocytosis, by the production of leukocytosis, a febrile reaction and other changes now so well known clinically as the "non-specific protein reaction."

Combination Chemotherapy and Biologic Therapy of Bacterial Diseases. This raises the question of the practical value of combining chemotherapy with such biologic therapy as the administration of immune serums, vaccines, non-specific protein agents and blood transfusion, in the treatment of bacterial diseases. All of my clinical experience indicates that best results are

to be obtained by the judicious use of both kinds of therapy in the treatment of bacterial diseases and especially the acute infections. Chemoserotherapy, or the combination of chemotherapy with serum therapy, has been before the profession for several years and is deserving of the closest study and attention.

After all, we are more interested in the recovery of our patient than in figuring out in exact terms to what recovery may be ascribed and I rarely depend upon chemotherapy alone in the treatment of the acute bacterial diseases, but always include serum therapy, blood transfusion or vaccine therapy when indicated, as will be discussed and presented in Chapter VIII, devoted to the practical chemotherapy of bacterial diseases. In other words the chemotherapy of bacterial diseases has not progressed by any means to the stage where we can afford to neglect surgery, biological therapy or any other helpful therapeutic means for winning the battle with infection. For example, I rarely neglect to use antistreptococcus serum in the treatment of acute streptococcus infections with septicemia along with gentian-violet or mercurochrome. I am likewise very partial to transfusions of blood and especially in the treatment of long continued and exhaustive infections associated with anemia. But I shall reserve for Chapter VIII a fuller discussion of this important subject, wishing here to merely direct attention to the important subject of judiciously combining chemotherapy with biologic therapy in the treatment of the bacterial infections. Even the administration of vaccines and non-specific agents may be of aid because of their administration may produce mild focal reactions about the diseased parts characterized by hyperemia and serous exudation which may facilitate the penetration of a chemotherapeutic compound in the blood and prove generally useful when due care is exercised in dosage to avoid excessive hyperemia and exudation. I also surmise that judicious tuberculin administration to produce *mild* focal reactions of this kind may ultimately prove a useful adjuvant to the chemotherapy of tuberculosis, since the hyperemia and exudation may encourage the much to be desired penetration of tubercles by tuberculocidal chemical agents brought to the infected tissues by the blood.

As I shall point out in Chapter VIII, the combination of antipneumococcus serum with optochin has yielded better results in the treatment of experimental pneumococcus infections of mice than either optochin or serum alone and I have observed similar results with antistreptococcus serum and gentian-violet and the serum and mercurochrome, in the treatment of experimental streptococcus infections of the lower animals. The same has been observed in the treatment of anthrax and other bacterial infections, so that the combination of immune serum therapy and chemotherapy is recognized as distinctly useful in the treatment of a number of acute bacterial diseases.

Possibilities of Chemotherapy in the Bacterial Diseases.—Finally I may conclude this chapter by a brief reference to the possibilities for developing a specific chemotherapy for the bacterial diseases. As will be pointed out in the succeeding chapters a great deal of useful work has been done with the dyes, heavy metals, quinin and a host of other substances and the results have not been entirely fruitless or lacking in encouragement. I by no means share the skepticism so frequently expressed in regards to the future of the chemotherapy of bacterial diseases if money and interested workers are available for the tremendous expense and labor required for advancing our knowledge. It is true that more, much more, advance has been made in the chemotherapy of protozoal and metazoal diseases and that the problems of bacterial diseases are apparently more difficult, but when one considers

the advances made during the past five years it is neither rash nor unwise to expect greater discoveries in the future as the fruit of hard, conscientious and unremitting, but systematized toil coupled with the ever alluring chance of making a "lucky strike." A larger appreciation of the possibilities of chemotherapy in the bacterial diseases should lead to a greater expenditure of brains and money as being warranted by the importance of the problems and the prospects of valuable results; as so well stated by the late Morgenroth:[4]

"What yesterday seemed absurd, tomorrow will seem banal. Today it is no longer a question for debate whether or not there can be a prophylactic and curative chemotherapeutic antisepsis, but merely a question of when, where and how. The future of chemotherapeutic antisepsis will probably resemble the present situation in respect to local and general anesthesia, for the future surgeon will inquire in each case whether the antisepsis should be local or be general through the circulation, which antiseptic is to be selected, what concentration and quantity, etc., just as he now does with his anesthetics."

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CHAPTER IV

METHODS FOR CHEMOTHERAPEUTIC INVESTIGATION IN BACTERIAL AND MYCOTIC DISEASES

The principles and technic of methods employed in chemotherapeutic investigations in bacterial diseases are subjects of considerable importance and deserving of closer attention and analysis than they have generally received. It is the general impression, for example, that bacteriostatic and bactericidal tests *in vitro* with new compounds are of little or no real value in relation to the actual treatment of disease; this, however, is far from being true although not infrequently new compounds, rushed into clinical use on the basis of tests *in vitro*, have yielded disappointments largely because the methods employed were woefully lacking in proper appreciation of essential principles in technic.

Much time and useless duplication of effort would be saved by the adoption of standard methods enabling different workers to better compare results, but at the present time almost every investigator in this field has employed a method or methods varying to a greater or lesser degree from the methods of others; this, however, is not nearly as important as the wider recognition of the essential principles involved as well as of the advantages, disadvantages and limitations of various methods. The very nature of chemotherapeutic research demands that there shall be available a variety of methods, but it is imperative that the purposes and principles of the methods are properly understood and appreciated in order to avoid the regrettable error of drawing unwarrantable conclusions and too hasty acceptance or condemnation of new compounds.

Classification of Experimental Methods in Bacterial Chemotherapy.—In the first place, it may prove helpful to classify the different tests and methods that may be employed in chemotherapeutic research in bacterial diseases.

Naturally the earliest and best known of these are various *in vitro* methods for determining the effects of a chemical agent upon bacteria under artificial conditions and two principal kinds are employed, namely, (*a*) *antiseptic or bacteriostatic methods* for determining the smallest amount of agent capable of restraining the growth of organisms over a given period of time and (*b*) *bactericidal methods* for determining the smallest amount of agent capable of killing organisms in a given period of time. Each has a useful place in chemotherapeutic research as will be shortly discussed, although the bactericidal tests are the more important and have a closer relation to the chemotherapy of bacterial diseases when arranged and conducted with a definite purpose in mind. For example, if a compound is being developed primarily for the local disinfection of wounds, tests of this kind should be conducted with a menstrum rich in the proteins and salts of the blood and muscles; if it is designed primarily for the treatment of infections of the urinary system, tests conducted with sterile urine are useful and if for systemic infections, the tests should be conducted with serum or, better still, with whole blood, etc. Bacteriostatic tests must be conducted with a suitable culture medium, since it is necessary that the organism shall survive and multiply unless restrained by the chemical agent under study, but the medium may also contain blood, serum, ascites fluid, muscle extract, etc.

In vivo methods are designed for testing out the effects of a compound in living animals infected with a particular organism. As a general rule, they are only conducted after the toxicity or organotropism of the compound has been determined and preferably for the same species of animal. Tests of this kind may be divided into two main varieties, namely, (*a*) *protective tests*, in which the drug is administered shortly before, with or shortly after inoculation and (*b*) *curative tests*, in which the infection is given a good start with the production of bacteremia and lesions of the fixed tissues before the drug is administered. Needless to state, the protective kind of test is usually more favorable to the compound than the curative test because the number of organisms requiring destruction is smaller and time is not permitted for the production of modifying pathological changes in the tissues.

Protective and curative tests may be conducted with either generalized (systemic) or localized infections. The former have been generally employed, but the latter, like pleuritis, arthritis, meningitis, dermatitis, etc., are sometimes especially useful although it may be difficult or impossible to produce experimentally a strictly localized infection of the serous cavities like those of joints, pleurae, pericardium or subarachanoid space.

Another type of *in vivo* test consists of administering the compound and testing the blood at intervals for increased bactericidal effects by removing small amounts under sterile conditions, seeding with a test organism and plating the mixtures after suitable intervals. This might be designated as a *combined in vivo and in vitro method*, and serves a useful function in chemotherapeutic research. The reverse of this method is also sometimes useful, consisting of adding the organism to the disinfectant agent *in vitro* and after a suitable interval injecting a portion of the mixture into a susceptible animal for determining the degree of bactericidal activity. This is likewise a *combined in vitro and in vivo method* which has been found useful in studies upon the chemotherapy of pneumococcus, streptococcus and other bacterial infections, as well as in chemotherapeutic investigations with pathogenic trypanosomes and spirochaetes.

All of these tests are governed by certain fundamental principles and possess certain advantages as well as disadvantages in relation to the subject of chemotherapeutic research and before the methods are described it may

be well to briefly summarize these subjects in order that their value and limitations shall be defined at the outset.

Value of Bacteriostatic and Bactericidal Tests in Vitro in Relation to Chemotherapy.—In the rational development of the chemotherapy of bacterial infections it is desirable, if possible, to commence experimental work with a substance or substances possessing some definite destructive effect on the microparasite under study. This parasitotropic effect may be apparent only in test-tube experiments; in such event, an effort is made to lower the toxicity or organotropic effect of the substance, with, or even without, an increase of its parasitotropic power, in order that it may be administered in such quantity as will exert in the living animal an inhibitory or killing action on the microparasite under study without injury to the host.

In chemotherapeutic studies on bacterial infections, experiments *in vitro* may be said to have a positive value in preliminary orientation in the development of leads and the study of new compounds as they are produced. Experimental data at hand tend to show that substances possessing a high bactericidal activity *in vitro*, and particularly in a menstrum of fresh sterile serum, are likely to exert an inhibitory effect *in vivo*. For example, Morgenroth's drug, optochin (ethylhydrocuprein), and its hydrochlorid exert a very high bactericidal action on the pneumococcus *in vitro* and are likewise effective to some extent *in vivo;* other cinchona derivatives, including certain salts of quinin, possessing more or less bactericidal value *in vitro* are likewise effective to a certain degree *in vivo*.

Unfortunately, however, numerous substances possessing a high degree of bactericidal activity *in vitro* and even in a menstrum of serum or blood, may fail to influence an infection *in vivo*. Indeed, since the time of the earliest discoveries in bacteriology and of bactericides, substances highly bactericidal in the test tube have been known to fail to exert any appreciable influence *in vivo* when given in safe doses. This lack of effect may be due to the high organotropic or toxic effect of the substance on the body cells in general or on a particular group of cells of vital centers, precluding the use of a bactericidal quantity; to insolubility and difficulty of administration; to rapid union with the proteins of the body and the formation of inert compounds; to rapid elimination; to failure to reach the microorganism in a lesion, and to still other causes. Of the possibilities the first mentioned is of primary importance; but the method and manner of attack of the bactericidal drugs on the protoplasm of cells, aside from coagulation, is almost unknown. Nevertheless, it is possible by systematic modification of the molecule through addition, removal, or substitution of certain atom groups, to produce in some instances a sufficient lowering of toxicity to give an available therapeutic agent. The demonstration of this fact constitutes the great triumph of Ehrlich and opens a fascinating field of research to chemist and biologist.

It is highly probable that experimental studies tending to increase the monotropism of a drug *in vitro* and particularly in a menstruum of serum will prove of value in chemotherapeutic work. On the other hand, a substance failing to exert parasitotropic action either *in vitro* or *in vivo* may still prove of value as a basis of composition, and offer a valuable lead in chemotherapeutic research. Likewise, it is possible that a chemical may be more efficacious *in vivo* than *in vitro* by reason of the formation of new and more active compounds *in vivo;* or by exciting the body cells to produce antibodies for the parasitic antigen or chemically facilitating such production as by the stimulation of phagocytosis or some other effect upon immunological processes.

It is highly probable, therefore, that experiments *in vitro* have a definite value in chemotherapeutic research as methods of preliminary orientation

and in the development of monotropic chemicals. This value is greater when the identical microparasite causing the definite infection under study is employed in the tests. In the absence of a pure culture of the particular microparasite against which a destroyer is sought, or in the presence of insuperable technical difficulties, other and more easily cultivated organisms closely or remotely related, or even of a different biologic order, may be employed.

Principles and Special Requisites of Bacteriostatic and Bactericidal Tests in Relation to Chemotherapy.—It is well known that many factors influence the results of bactericidal tests, and many investigators, notably Rideal and Walker,[1] and Anderson and McClintic[2] have sought to establish standard methods for the bacteriologic standardization of disinfectants. Consideration of the physical and chemical laws underlying the action of chemicals on bacteria *in vitro* are to be found in their papers, and in that of Phelps,[3] Chick[4] and others, the work of Miss Chick being especially valuable for showing that in all essential particulars the act of disinfection may be regarded as obeying the laws governing a simple chemical reaction, in which the disinfectant represents one reagent and the bacteria the other.

For chemotherapeutic studies two main kinds of *in vitro* tests may be employed: (1) a simple technic observing the first principles governing these reactions, particularly a uniform temperature, and employing an easily cultivated microorganism, as *B. typhosus* or *Staphylococcus aureus;* or (2) a technic aiming at conditions more closely approximating those operative in the living animal.

As a result of a large amount of work with various methods, one fact stands out clear, and that is the necessity of adopting a uniform technic. The most marked variations in bactericidal power appear as the test methods employed are varied. For this reason Cohen, Heist and I[5] have studied various methods quite extensively and those herein described are partly based upon the results of our work with the pneumococcus.

With certain microörganisms, as *B. typhosus* and staphylococci, either the Hygienic Laboratory or the Rideal-Walker method may be employed to determine the bactericidal action of new compounds developed in the course of chemotherapeutic studies. But these methods are not adapted for work with microörganisms less hardy and requiring special culture media, as the pneumococcus, gonococcus, and streptococcus. Furthermore, it has appeared to me that for the special purposes of chemotherapeutic study an acceptable method should approach as nearly as possible the conditions presented in the living animal, particularly with reference to temperature.

Here may be summarized what may be considered the essentials of an acceptable *in vitro bactericidal test* for the special purpose of chemotherapeutic studies in bacterial infections:

1. The test should be conducted at a temperature of 37°C. instead of 20°C. in order to approach more nearly conditions in the living animal. The influence of temperature on the chemical reaction between bacterium and bactericide has been uniformly emphasized and constitutes a very important factor. The velocity of disinfection is known to increase with rise in temperature in a manner similar to that of an ordinary chemical reaction.

2. The solution of chemical should contain some organic matter, as the protein of broth, sterile inactivated serum, or ascites fluid. Wide variations in bactericidal power are noted according to whether the menstruum is serum, pus, or salt solution, as will be shortly discussed.

When large numbers of new compounds are to be tested at frequent intervals, and particularly in an effort to build up a monobacteriotropic chemical, the simpler technic fulfilling at least the fundamental laws of chemical reaction and utilizing sterile water or salt solution as the menstruum, may be employed; but when an effort is being made to obtain from *in vitro* tests data of greater value in relation to the probable effect of a given parasitotrope *in vivo*, serum or blood are to be preferred.

3. In the chemotherapeutic study of a definite infection the causative microorganism should be employed whenever possible.

It would also appear advisable to employ a freshly isolated culture from human or experimental lesions in the conduct of certain tests which aim to be conclusive. This has been found especially true of the pneumococcus. The presence or absence of capsules and the degree of virulence of the strain for white mice and rabbits appear to influence markedly the results of tests *in vitro*. For routine tests, however, it is better to employ a uniform and known culture or cultures well adapted for growth under artificial conditions, but preferably retaining some degree of virulence.

4. It is necessary to employ in the tests a sufficient number of microörganisms to yield well-defined results. In work with the pneumococcus and similarly less hardy bacteria, appropriate culture media must be employed and frequent counts made by the plating of twenty-four hour cultures to determine the approximate numbers of living microorganisms present.

Tests may be conducted after ordinary methods with low concentrations of the chemical over long periods of time, with apparent inhibition of the microorganism which may amount to true killing (the "bacteriostatic" test); or with higher concentrations but shorter periods of exposure, and a definite attempt to determine whether or not killing has resulted (the "bactericidal" test). Plating methods attempt to measure the degree of bactericidal activity after a definite exposure to varying concentrations of the chemical, according to the number of bacteria killed.

As pointed out by Phelps and amply confirmed by Cohen, Heist and myself, bacteriostatic power, or true disinfection at low concentrations for long periods, may show a relation between two chemicals quite different from that shown at higher concentrations and shorter periods of exposure. One compound may be a superior bactericide in high concentration and lose rapidly in efficacy with dilution and hence prove to be a poor antiseptic; while the other, though of lesser value in high concentration, retains its power better with dilution.

Various Menstrums for Bacteriostatic and Bactericidal Tests in Vitro in Relation to Chemotherapy.—The selection of the menstrum or medium with which the disinfectant and organism are brought together is a matter of considerable importance. Almost without exception a medium of serum, blood, muscle extract, etc., greatly reduces germicidal activity in both short and prolonged exposures. As stated by Dakin and Dunham[6] the capacity of a disinfectant to kill organisms is dependent to an extraordinary degree upon the conditions under which it acts. Almost invariably the greatest antibacterial activity is shown when the substance acts upon bacteria freed from culture media and suspended in distilled water or salt solution. They have found the activity of mercuric chlorid against staphylococci in a hard tap water to be only one-fifth of that shown when distilled water was employed, while with silver salts the results were even more liable to variation.

The presence of 50-per cent. serum reduces bactericidal activity approximately 5 to 200 times depending upon the compound. In the presence of pus, in which many bacteria are partly infested in the bodies of the dead leukocytes and hence difficult to attack, the results are still less favorable as shown by Morgan[7] and others.

It is stated that some derivatives of hexamethylenetetramin are less affected by serum and according to Browning[8] one compound known as trypaflavin or acriflavin is about ten times more bactericidal in serum than in peptone solution. I have observed that 5 c.c. of a final dilution of 1 : 800 of acriflavin in 50 per cent. horse serum killed staphylococci (1 c.c. of 18 hours broth culture) in fifteen minutes at room temperature while 1 : 500 in sterile peptone water gave the same result. After an exposure of one hour, however, the results were almost identical, being about 1:800 in both menstrums.

The selection of the menstrum or medium for tests of these kinds is largely controlled by circumstances and according to the kind of information desired. For routine purposes and to determine bacteriostatic and bactericidal activities under the most favorable conditions, either plain nutrient broth, saline or Locke's solutions may be employed; if however, the disinfectant is designed for use in the treatment of infected wounds or serous cavities it is advisable to employ menstrums of muscle extract, serum or pus; if designed for administration by intravenous injection, menstrums of blood or serum are advisable; if designed for use in the genito-urinary tract by local application, a menstrum of sterile urine, etc., may be employed. In other words the choice of medium should be influenced by our therapeutic designs for the agent under study and one should carefully avoid basing conclusions on the results of tests employing saline solution or water, as bearing upon the disinfection of an infected wound, an infected serous membrane, the blood, etc.

Due care must be also exercised in guarding against the selection of a menstrum in itself bactericidal. Thus some organisms may perish in plain distilled water and especially if the exposure is prolonged; some organisms like the meningococcus may even perish in saline solution. Fresh defibrinated blood and fresh serum may prove in themselves slightly bactericidal and especially for organisms of the colon-typhoid group. It may be necessary, therefore, to keep these factors in mind when working with certain organisms.

Phenol and Mercuric Chlorid Coefficients.—So many factors may appreciably influence bacteriostatic and bactericidal tests *in vitro* that it is almost hopeless to expect different workers to obtain identical results. The slightest change in the reaction of the culture medium and indefinable changes in the test organism are the chief sources of variation. Only in the simplest methods and by strict attention to every detail including the use of the same culture, may different workers obtain approximately the same results as with the Rideal-Walker or Hygienic Laboratory phenol coefficient methods.

For these reasons I believe that a control and standardized disinfectant like phenol or mercuric chlorid should be used in every bacteriostatic or bactericidal test and the results observed with a new compound expressed in terms of comparison with the standards. For this purpose, a titrated and standardized 5-per cent. solution of pure phenol may be employed for preparing dilutions; I believe this standard is well adapted for tests with chemical compounds of relatively low bactericidal activity. For testing new compounds of mercury, copper, silver, etc., possessing a higher bactericidal activity *in vitro*, a chemically pure mercuric chlorid is advisable. This substance apparently does not change if properly sealed and furnishes a fixed standard for comparative purposes.

For example, the highest bacteriostatic dilution of a new mercurial compound may be 1:800,000 and bichlorid 1:480,000; the *bichlorid index* or *coefficient* would be expressed as plus 1.66. When these substances are tested again at a later time in apparently the same manner and using the same organism, the bacteriostatic dilutions may be different like 1:1,000,000 and 1:650,000 respectively; however the bichlorid index is plus 1.5 which is about as near as one may expect in work of this kind. A new compound may be bacteriostatic in 1:80,000 and bichlorid in 1:400,000; the index in this case is expressed as minus 5.0, the plus sign indicating that the new compound is more and the minus sign that it is less, in bacteriostatic or bactericidal activity than mercuric chlorid. Phenol may be used in the same manner. I have used this method for over ten years in bacteriostatic

and bactericidal tests and for expressing results and can highly recommend it, because the factors tending to raise or lower the titers of a new compound like variations in the culture medium or organism, exert the same influence upon the control bichlorid or phenol and thereby yield approximately the same coefficients or indices.

Technic of in Vitro Bacteriostatic Methods.—The purpose of bacteriostatic tests is to determine the highest dilution of a chemical agent capable of restraining the growth of the test organisms for a stated period of time; this may finally amount to a complete destruction of all of the organisms and especially in the lower dilutions while in the higher dilutions only proliferative activities or genesistasis is inhibited, the organisms being found alive when removed by subculture. Tests of this kind are much simpler than bactericidal tests and the results are usually very sharp and definite; I can highly recommend them for routine work and especially when it is desired to ascertain if a new compound displays any specific parasitropism for a particular kind of organism and how it compares with either phenol or mercuric chlorid in any stated medium or several mediums. The technic is so simple that tests may be conducted simultaneously with several organisms or several mediums for routine studies.

Author's Method Employing Nutrient Bouillon.—For tests with staphylococci, *B. anthracis*, *B. typhosus*, *B. coli* and such hardy organisms, plain beef extract broth with a pH of 7.1 may be employed; Kligler has used a medium prepared of 1-per cent. Fairchield's peptone, 0.5-per cent. dibasic potassium phosphate, 0.5-per cent. sodium chlorid and 0.1-per cent. glucose with a constant pH of 7.1. The reaction of any medium employed is particularly important as the results may be greatly modified by this factor. For streptococci and pneumococci harmone broth (Huntoon) with 0.1-per cent. dextrose and a pH of 7.7 is to be preferred and for tubercle bacilli, the ordinary 5-per cent. glycerin harmone broth employed in the manufacture of Koch's tuberculin should be used. *It is always advisable to determine beforehand that the organism will grow well in the medium employed before the tests are conducted.*

(1) As a general rule, 10 dilutions are advisable and for this purpose 10 sterile test tubes are arranged for each compound including a set for the bichlorid of mercury or phenol controls.

(2) In all tubes except No. 1 of each series, place 1 c.c. of sterile distilled water.

(3) In tubes 1 and 2 place 1 c.c. of the stock solution of disinfectant which is 10 times higher than the final dilutions desired. Mix No. 2 and transfer 1 c.c. to No. 3 and so on to No. 10 from which discard 1 c.c.

(4) In a flask of 99 c.c. of the culture medium place 1 c.c. of a twenty-four to forty-eight hour broth culture of the test organism; with such organisms as streptococci and pneumococci it is well, however, to seed by mixing 5 c.c. of a broth culture with 95 c.c. of the culture medium. Mix well and add 9 c.c. to each tube of the set; the remaining 10 c.c. is placed in a sterile tube as a control on the culture.

(5) In tests employing tubercle bacilli, it is better to add 9 c.c. of sterile medium to each tube and then to seed by floating a loopful of bacilli on the surface of each.

(6) The final dilutions are now 10 times higher in each tube; for example, 1 c.c. of 1:1000 stock solution in No. 1 becomes 1:10,000 and the final dilution in No. 10 is 1:5,120,000.

(7) The tubes are incubated and the results recorded daily for five days. When the medium remains clear the result is recorded as *minus;* when a visible growth appears the result is recorded as *plus*. At the end of this period the tubes may be cultured by transferring several loopsful to slants of a solid medium to determine whether the organisms have been killed or merely restrained. In this manner a bactericidal test is conducted at the same time in which a few organisms have been exposed to the disinfectant for five days. The control should be subcultured at the same time to make sure that the organisms are viable.

(8) The results are expressed according to the highest bacteriostatic and bactericidal dilutions and also according to the bichlorid or phenol coefficients previously described.

(9) After obtaining in this manner an approximate idea of the activity of the compound under study, a second series of dilutions are prepared in which the variations from tube to tube are less marked.

Author's Method Employing Serum, Blood, Ascites Fluid or Muscle Extract.—These tests are conducted in exactly the same manner as described above except that to 89 c.c. of a suitable broth medium are added 10 c.c. of sterile serum, blood or ascites fluid; the mixture is

then seeded with 1 c.c. of a broth culture of the test organism; with such organisms as the pneumococcus and streptococcus however, it is generally advisable to use 8.5 c.c. of both, 10 c.c. of serum, blood or ascites fluid with 5 c.c. of broth culture of the test organism. This gives a 10-per cent. solution of serum, blood or ascites fluid and while more or less may be employed as desired, yet experience has indicated that the above is satisfactory for eliciting the influence of these substances upon the degree of antibacterial activity of disinfectants.

Numerous experiments have shown that while the bacteriostatic and bactericidal activity of various disinfectants is reduced to a greater extent on a 50 per cent. than a 5-per cent. dilution of serum, defibrinated blood, or ascites fluid, yet for all practical purposes a 10-per cent. solution is satisfactory for this purpose and in view of the large amounts required is to be preferred from the standpoint of economy. With muscle extracts, however, it may be advisable to use equal parts with broth (50-per cent.), but muscle extract may be prepared so cheaply and quickly as required that the questions of economy and supply are not involved. These preparations are likewise slightly cloudy but not usually to a degree sufficient for interfering with the ease and accuracy of readings. I may add in this connection however, that a menstrum containing 10-per cent. of a muscle extract prepared of ordinary beef or veal requires a far higher concentration of disinfectants than a menstrum of 10-per cent. blood or serum; this is doubtless due to the presence of large numbers of various bacteria in addition to the test organism.

Other special mediums like harmone dextrose broth with brain tissue, ascites broth with sterile kidney, etc., may be employed in tests of this kind and while the results vary according to the constitution of the medium, yet if mercuric chlorid or phenol are included in each and every test, the results may be expressed in terms of the coefficients or indices. The coefficients, however, will vary according to the chemical nature of the compound as this is influenced by the constitution of the medium; for example, some compounds of mercury like mercurophen and metaphen, maintain a higher degree of bacteriostatic activity in a serum, blood or brain medium than mercuric chlorid and thereby yield higher coefficients. In other words while the bactericidal activity of almost all disinfectants is reduced in the presence of serum, blood, muscle extract, etc., the degree of reduction varies considerably among different compounds.

Author's Method Employing Solid Mediums; Mycostatic Test.—It is very easy to employ solid culture mediums in this technic and for such organisms as grow better on solid than fluid medium, as the various yeasts and molds, the former are to be preferred.

In conducting this test, a series of dilutions of the disinfectant in amounts of 1 c.c. in sterile distilled water are prepared in sterile test tubes as previously described. To each tube and a control are now added 9 c.c. of an appropriate agar medium cooled to 42°–45°C.; the contents are well mixed and allowed to harden in slants. The dilution in each tube is now ten times higher; or 4 c.c. of medium may be added to each tube which renders the final dilution in each tube 5 times higher.

For such organisms as staphylococci, *B. typhosus*, *B. coli*, etc., plain 2-per cent. agar (pH 7.1) may be employed; for streptococci and pneumococci a harmone dextrose (0.1-per cent.) agar with a pH of 7.7 is to be preferred. For tubercle bacilli a glycerine agar may be employed since an egg medium may be unsatisfactory because heating for inspissation and sterilization may break up some disinfectants. For parasitic molds like *Trichophyton rosaceum*, *Microsporon audouini* and *Achorion schoenleinii*, Sabouraud's maltose medium titrated to 1-per cent. acid to phenolphthalein, may be employed. Whatever medium is chosen, it must be adapted for cultivating the test organism, should be sterile, liquified by heating and added to the tubes containing varying dilutions of the disinfectant while still fluid after being cooled to 40°–45°C. After hardening has occurred, each tube is inoculated in as uniform manner as possible and the tubes incubated for a period of 5 to 10 days (mycostatic tests for 2 weeks or longer at room temperature) as decided upon. The results are expressed according to the highest dilution of disinfectant capable of preventing the growth of the test organism. Mercuric chlorid or phenol controls may be included and the results expressed in terms of coefficients.

Technic of in Vitro Bactericidal Methods.—From the standpoint of chemotherapy the results of bactericidal tests probably possess a more direct value and application than bacteriostatic tests because the duration of disinfection of the living tissues is almost always a process of short duration. A very large number of different methods have been proposed from time to time but the older "thread" method of Koch and the "garnet" method of Kronig and Paul are not now generally used. No single method can serve as a means of comparing the value in practice of disinfectants of greatly diverse composition and destined for a variety of applications, but as

a general rule a satisfactory method may be easily devised to meet special conditions if the primary and fundamental principles are kept in mind.

For disinfectants chemically related to phenol, which are to be used against organisms reacting similarly to the manner in which the typhoid bacillus reacts and which are destructive within fifteen minutes at 20°C., the Rideal-Walker or Hygienic Laboratory methods may be employed. The results may be useful in the selection of a potent product, in making comparisons of cost in terms of service rendered, and in checking successive batches of the same product. I shall omit here the technic of the well known Hygienic Laboratory method as recently described[9] and proceed with the description of methods which I have found of more value in relation to chemotherapeutic investigation in bacterial diseases.

Author's Bactericidal Tube Method.—The principle of this method is to determine approximately the highest dilution of disinfectant proving completely bactericidal for large numbers of organisms in exposures of 5, 30 and 60 minutes at 38°C. The method is adapted for conducting tests in any menstrum as water, broth, serum, blood, ascites fluid, muscle extract, urine, etc., and the intervals of exposure may be modified at will.

1. For each disinfectant arrange 10 sterile test-tubes, the first 9 carrying 1 c.c. of varying dilutions in sterile distilled water 5 times stronger than the desired final concentrations; the tenth tube carries 1 c.c. of sterile water for a control.

2. In a small sterile flask or glass stoppered cylinder place 10 c.c. of the chosen menstrum as sterile distilled water, saline, broth, serum, ascites fluid, defibrinated blood, urine, muscle extract, etc. Add 20 c.c. of sterile distilled water and mix.

3. Add 10 c.c. of a twenty-four hour plain meat extract or harmone broth culture of the chosen organism filtered through sterile paper (as *B. typhosus*, *B. para-typhosus*, *B. coli*, *B. pyocyaneus*, *B. prodigiosus* or *Staphylococcus aureus*). Streptococci and pneumococci may be cultivated in 0.1 per cent. dextrose harmone broth but the addition of serum or a tissue (brain) should be avoided if the tests are to be conducted in a plain menstrum. Otherwise these organisms and *B. tuberculosis* may be cultivated on a solid medium or centrifuged out of a fluid medium and a homogenous suspension in plain broth or saline solution prepared containing at least one hundred million organisms to the cubic centimeter. As a general rule a suspension of *B. anthracis* of this kind is better than a broth culture. The suspension should be shaken with sterile beads for at least one-half hour to break up clumps (tubercle bacilli should be first triturated in a sterile mortar) and finally filtered through a rather coarse fat free sterile paper.

4. Mix well and add 4 c.c. to each of the 9 tubes carrying varying dilutions of disinfectant and the balance to tube No. 10 which is the culture control.

The final dilutions of disinfectant in each tube are now five times higher; for example, 1 c.c. of a 1:100 solution in tube No. 1 now has a final dilution of 1:500 acting on the organisms in a 20-per cent. solution of the chosen menstrum (water, saline, broth, serum, blood, muscle extract, etc.). The tubes should be plugged in the usual manner to prevent air contamination.

5. Note the exact time when the mixtures are made; keep the tubes in a water-bath at 38°C. and at intervals of 5, 15 and 60 minutes subculture two loopfuls to either slants of appropriate solid medium or to tubes of a suitable fluid medium (10 c.c. in each), the choice of medium depending upon the organism being employed (as plain extract broth or agar for staphylococci, typhoid, colon, and anthrax bacilli; egg medium for tubercle bacilli; blood agar or serum dextrose broth for streptococci, pneumococci, etc.). Instead of seeding with a platinum loop it is permissible to use a capillary pipet for transferring 1 or 2 drops, the pipet being sterilized between transfers by several washings with boiling water.

6. Include a series of tubes carrying dilutions of phenol or mercuric chlorid as controls for expressing the results in comparison with these disinfectants.

7. The test may be shortened by subculturing at any one interval that may be chosen as at the end of 2, 5, 10, 15, 20, 30 minutes or longer periods; for exposures of two hours or more the incubator may be employed instead of a water bath.

8. Incubate the subculture seed tubes for 24 to 72 hours (10 days or longer for tubercle bacilli) and record the results if the culture control has shown a satisfactory growth.

9. The results are expressed according to the highest *final* dilution of disinfectant proving completely bactericidal at any one, all three or more of the time intervals chosen; for any one or all of these intervals the results may be also expressed according to phenol or mercuric chlorid coefficients.

Author's Bactericidal Plating Method.—The primary purpose of this method is to determine the rate of bactericidal activity over any period of time in any chosen menstrum and preferably at 38°C. It is advisable to know beforehand the approximate activity of the

compound under study which may be determined by the tube method described above. The test is only applicable for such organisms as may be readily plated out in plain or serum agar.

1. For tests employing *Staphylococcus aureus, B. typhosus, B. coli, B. anthracis* and such organisms as grow readily in plain agar, five tubes containing 10 c.c. each of plain or harmone agar should be boiled and kept in a water bath at 45°C. For streptococci and pneumococci a serum harmone agar may be prepared by adding 1 c.c. of sterile serum to tubes of harmone agar after they have been boiled and cooled to 45°C.

2. Arrange five sterile petri dishes.

3. In a sterile test tube place 2.5 c.c. of sterile distilled water, 1 c.c. of the chosen menstrum (sterile water, broth, serum, defibrinated blood, urine, muscle extract, etc.) and 0.5 c.c. of broth culture or suspension of the chosen test organism prepared as described above for the tube method.

4. Place the mixture in a water-bath at 38°C. for several minutes and then add 1 c.c. of a solution of the disinfectant in sterile water 5 times higher than the chosen final solution. For example, 1 c.c. of a 1:200 solution would give a final dilution of 1:1000. Use a sterile 1 c.c. pipet and wash by drawing in and expelling the mixture several times, noting the time when the organisms are added. It is always imperative to add the disinfectant last and especially when labile substances like chlorin antiseptics are being tested for otherwise the rate of chemical reaction taking place between the unstable antiseptic and organic menstrum may be a more important factor than the rate of bactericidal activity of the substance under study. Mix the contents and plug the tube in the usual manner to prevent contamination.

5. In a second tube place 1 c.c. of the culture, 1 c.c. of the menstrum and 3 c.c. of sterile distilled water; mix, plug and place in the water bath. This is the culture control.

6. At chosen intervals like 5, 15, 30 and 60 minutes place 0.05 c.c. of the mixture carrying disinfectant in a sterile petri dish. For this purpose a sterile 1 c.c. pipet may be used, being washed out between transfers with boiling water. Instead of these intervals one may chose 1, 15, 30 and 60 minutes or 5, 30, 60 minutes and 2 hours, etc.

7. Add a tube of the agar medium and mix thoroughly. After hardening the plates are incubated upside down for forty-eight hours and the colonies counted in the usual manner.

8. The control is cultured in the same manner at the end of 60 minutes. However, plates may be prepared at the same intervals as the disinfectant mixture; this would require the preliminary preparation of three more petri dishes and three more tubes of culture medium.

9. One drawback to all plating methods is the possibility of carrying over to the plates a sufficient amount of the disinfectant to produce bacteriostasis. For example, if a final dilution of mercuric chlorid 1:1000 or some equally potent bactericide were being tested in a plain menstrum, plating 0.05 c.c. with 10 c.c. of agar would give a final dilution of approximately 1:200,000 which may prove sufficiently bacteriostatic for yielding very erroneous results. This error may be avoided by using the highest final dilution of disinfectant likely to be completely bactericidal in one hour as determined in a preliminary tube test in order to carry over the minimum amount of disinfectant. Otherwise, an antidote may be added to each petri dish at the time of plating to neutralize the disinfectant, as 1 c.c. of a sterile 1-per cent. solution of sodium thiosulphate for chlorin disinfectants or a similar dose of potassium sulphid for salts of the heavy metals.

Author's Combined Test-tube and Animal Bactericidal Method.—This method has proven serviceable for determining the bactericidal activity of disinfectants for such organisms as pneumococci, streptococci and anthrax bacilli virulent for white mice by intraperitoneal inoculation. It has the distinct advantage of controlling the result by animal inoculation, which in the case of a highly virulent culture of a pneumococcus constitutes in my experience a delicate test for viable microörganisms. The disadvantage of the test lies in the fact that in such a combined *in vitro-in vivo* method, some of the drug is injected intraperitoneally and probably continues its bactericidal activity within the peritoneal cavity for a short time at least.

In this method 1 c.c. of a dilution of the bactericide in serum, broth, or salt solution is allowed to act on 1 c.c. of a culture of pneumococci or other organism of known virulence so diluted that 0.1 c.c. contains 100 minimal lethal doses, at a temperature of 37°C.; at intervals of 5, 15, 30, 45, and 60 minutes, 0.2 .cc. of the mixture is injected intraperitoneally into mice to determine when killing of the pneumococci has occurred.

1. A twenty-four-hour broth culture of a pneumococcus or other organism is titrated by intraperitoneal injection into mice to determine the M.L.D. in a period of 24–36 hours after injection. This culture is then diluted with sterile broth until 100 times the M.L.D. is continued in each 0.1 c.c.

2. A dilution of the bactericide in serum, broth, or salt solution is prepared—for example, 1:1000 ethylhydrocuprein hydrochlorid—and 1 c.c. placed in a small sterile

tubule in a water-bath at 37°C. After a few minutes 1 c.c. of the diluted culture is added and the contents gently mixed. The dilution of bactericide has now been doubled (1:2000).

3. At intervals of 5, 15, 30, 45, and 60 minutes, 0.2 c.c. of the mixture is injected into the peritoneal cavities of white mice. The 1-c.c. graduated syringe used for this purpose is sterilized before each injection.

4. One or more controls are included by mixing 1 c.c. of the diluted culture with 1 c.c. of the same fluid used in preparing the dilution of disinfectant (serum, broth, or salt solution); the tubule or tubules are kept in the water bath and 0.2 c.c. injected into a series of mice at the same intervals.

5. The mice are kept under observation for at least six days, and at autopsy the blood of the heart is examined by smear and culture for organisms. During the first forty-eight hours death is generally due to bacteremia; after this time the blood is usually found sterile and death is ascribed to the toxicity of the drug injected.

Author's Methods for Determining the Bactericidal Activity of the Blood and Urine in Vitro, Following the Administration of a Disinfectant.—In the course of chemotherapeutic investigations it is sometimes useful to determine whether or not and for how long one may be able to detect an increased bactericidal activity of the blood or urine for a certain organism, presumably due to the presence of a chemical agent generally administered by intravenous injection. Various methods have been described from time to time and I have employed the following with considerable success and satisfaction.

As a general rule, experiments of this kind are conducted with rabbits because of the ease with which blood may be obtained from the veins of the ear. Large animals should be chosen and it is well to prepare one of the ears by shaving away the hair over the marginal veins. It is surprising, however, the frequency with which blood may be obtained without contamination by ordinary cleansing with soap and water followed by alcohol and ether. These manipulations usually render the marginal veins quite prominent and by pricking with a large sterile needle a good flow of blood is obtained. The various steps are as follows:

1. Prepare the ear as described. In each of a series of five small sterile test tubes marked off at the 5 c.c. level, place 4 c.c. of a sterile 0.2 per cent. solution of sodium citrate in saline. Collect 1 c.c. of blood in Tube No. 1.

2. Inject the compound by way of a vein in the opposite ear.

3. At chosen intervals like 5, 30 and 60 minutes collect 1 c.c. amounts of blood in Tubes 2, 3 and 4.

4. In the fifth or culture control tube place 4 c.c. of sterile broth.

5. Keep the five tubes in a water bath at 38°C. and in the meantime boil ten tubes of agar medium (10 c.c. in each) and cool to 45°C. Prepare ten sterile petri dishes.

6. Inoculate each tube with one 4 mm. loopful of a 24–48 hour broth culture of the chosen organism like *B. coli*, *staphylococcus aureus*, etc.

7. Mix well and 5 minutes later plate 0.05 c.c. of each mixture with 10 c.c. of agar at 45°C.

8. At the end of one or two hours prepare a second series of plates in the same manner.

9. After hardening incubate the plates upside down for twenty-four to forty-eight hours and count the colonies. The first or preliminary specimen of blood shows whether or not it is alone bactericidal but owing to the 1:5 dilution this is not usually the case even with typhoid and paratyphoid bacilli. The culture control plates usually contain a very large number of colonies and especially those poured at the end of one or two hours owing to proliferation of the organisms at 38°C.; if, however, the plates of post-administration blood prepared one or two hours after inoculation contain no more organisms than the plates prepared five minutes after inoculation, it may indicate that the blood contained bacteriostatic amounts of disinfectant.

The method is also suitable for measuring the bactericidal activity of the blood of human beings following the administration of optochin, mercurochrome and other substances in the treatment of disease. As a general rule, with rabbits only the maximum tolerated doses of such compounds as ethylhydrocuprein hydrochlorid show a distinct increased bactericidal activity of the blood for the pneumococcus, mercurochrome for *B. coli*, etc.

A second method which I have used with some success is an adaptation of the test devised by Heist, Cohen and Cohen[10] for measuring the bactericidal activity of whole coagulable blood. The essential feature of the method is that a capillary glass tube is filled, by capillary attraction, up to a fixed mark, with a broth culture of the bacterium, and then emptied. A certain number of bacteria remain sticking to the wall of the tube. Blood as it comes from capillary or vein is allowed to flow up the tube to the mark, and the tube is then sealed and incubated. If the blood has no bactericidal action the bacteria which have remained on the wall of the tube find themselves in a favorable medium and multiply rapidly; if it is bactericidal, they are killed and no growth results. Readings

are made by blowing out the contents of the tube on a glass slide, staining and examining it under the microscope. By combining several capillary tubes into one many-stemmed pipet, modeled after the one Wright uses for estimating the coagulation time of the blood, and by using a series of ascending dilutions of broth culture, an approximate quantitative value may be given to the test. Borow and myself[11] have employed this method with some success in measuring the bactericidal activity of the blood after the administration of ethylhydrocuprein hydrochlorid and I have described elsewhere[12] the details of the technic as well as the Stern and Korte plating and Wright capillary tube methods, all of which are likewise adaptable for measuring the bactericidal activity of the blood of human beings or the lower animals following the administration of disinfectant compounds.

Urine tests are best conducted with rabbits although it is very difficult to obtain uncontaminated specimens by catherization even under rigid aseptic conditions. The general plan of procedure is to catherize a male or female rabbit as carefully as possible to secure a control specimen of urine. The drug is then administered and at chosen intervals the animal is catherized for additional specimens. Each urine is then tested by placing 1 c.c. in a sterile test-tube in a water-bath at 38°C. and adding one 4 mm. loopful of a broth culture of the test organism (usually *B. coli* or a staphylococcus). An additional control should be included in which 1 c.c. of sterile water is used instead of urine. At the end of a chosen interval (usually 15 minutes) 4 c.c. of sterile water are added to each tube, mixed well, 0.05 c.c. transferred to a sterile petri dish and plated with 10 c.c. of agar at 45°C. After incubation for forty-eight hours the colonies are counted and the results evaluated in comparison with the controls.

Principles of Experimental Bacterial Infections in Relation to Chemotherapy.—But while bacteriostatic and bactericidal tests *in vitro* may be and usually are of distinct value in developing the chemotherapy of bacterial diseases, properly conducted *in vivo* tests employing systemic and localized experimental infections of the lower animals are usually the final tests for new compounds and if possible should be conducted before a substance is used or recommended for the treatment of diseases of human beings. It is true that experiments of this kind are usually very laborious and expensive and that it may be indeed difficult and even impossible to duplicate exactly the conditions occurring in the course of a disease in human beings for which the compound is designed but yet satisfactory experimental lesions can usually be produced with many of the pathogenic organisms for *in vivo* tests.

1. In my opinion, the first essential principle is to *avoid unnecessarily severe infections* because these may readily mask the feeble but definite curative effects of a compound under study. In serum and chemotherapeutic investigations in bacterial infections, animals are usually infected by subcutaneous or intraperitoneal injections of the test micro-organism resulting in the production of generalized infections associated with bacteremia and terminating fatally in a few days, or in a few weeks, as in experimental tuberculosis. As a general rule, the test animals are very susceptible to the test micro-parasite, and the results are evaluated by the duration of the lives of treated animals as compared with infected but untreated controls.

In experimental serum therapy of such bacterial infections as are produced by virulent pneumococci, streptococci, meningococci, anthrax bacilli, and so forth, this method is generally satisfactory because, owing to the low toxicity of the immune serums for the test animals, relatively large amounts may be given to influence favorably an otherwise rapidly fatal bacteremia and septicemia. But in chemotherapeutic studies in bacterial infections the *dosis curativa* of a medicament is likely to be but slightly less than the *dosis tolerata* or the *dosis lethalis*, and rapidly fatal and generalized infections do not disclose possible, although feeble, curative effects on the part of the compound under study, and thereby fail to yield information of value for the conduct of chemotherapeutic investigations.

For example, the maximum tolerated dose of ethylhydrocuprein hydrochlorid (optochin) for mice, by intravenous and intraperitoneal injection, is approximately from 20 to 40 mg. per kilo of body weight. When these animals are infected with 50 minimum lethal doses of Type I pneumococci, Cohen, Heist and myself[13] found that at least 20 mg. of drug per kilo must be administered at about the same time as the cocci to prolong the lives of approximately 20 per cent. of animals for an indefinite period. This amount was one half or more the highest tolerated dose and indicates the very small margin between *dosis tolerata* and *dosis curativa* of ethylhydrocuprein hydrochlorid for mice with pneumococcus bacteremia. Moore[14] has observed somewhat better results than we in the treatment of pneumococcus

bacteremia of mice with the ethylhydrocuprein base, but the margin between the toxic and curative doses was likewise small.

It is of course necessary to produce experimental lesions of such severity that untreated controls may not recover by natural means but we should carefully avoid producing overwhelmingly severe infections with rapidly fatal bacteremias.

2. A second principle is to *select an animal possessing some degree of natural resistance for the test organism.* For example, white mice are so susceptible to virulent pneumococci that it is very difficult to gauge a dose by intraperitoneal injection permitting the animal to survive for four or five days; white rats and guinea-pigs are more resistant and with a virulent culture may yield a longer experimental infection. Rabbits are so susceptible to virulent streptococci that a meningitis cannot be ordinarily produced of sufficient duration because of rapidly fatal bacteremia; cats and white rats are more resistant and offer much better chances for producing localized infections. In brief chemotherapeutic studies of bacterial infections demand that the infection shall not be too severe or too short in duration before a fatal issue. In the first place infections of human beings are rarely as severe as many of our experimental lesions and in the second place the toxic effects of the drug tends to still further depress resistance and favor the infection. We should aim, therefore, to conduct our experiments with animals possessing some degree of natural immunity for the test organism in order that there may be sufficient time to give several doses of the drug rather than to base results upon the administration of a single large dose which may actually shorten the lives of the animals as compared with untreated controls.

3. Indeed, I believe that this practically amounts to a third principle, namely, that *the infection shall permit of the administration of multiple doses of a drug* since there is but little hope for producing compounds capable of sterilizing completely in one dose, as arsphenamin and its allied compounds may accomplish in experimental trypanosomiasis and spirochetoses. Such may demand that we infect our animals with multiple small doses of organisms in order to produce a continuous, but milder infection ultimately fatal for untreated controls, and, indeed, during the past two years Dr. Harkins and I have developed a technic of this kind which has proven particularly serviceable for chemotherapeutic investigations of bacterial infections, as I shall shortly describe with more detail.

4. A fourth principle is to *give preference to the production of localized lesions rather than the production of almost immediate bacteremias.* For example, I believe it is much better to inoculate virulent streptococci into mice by subcutaneous injection as developed by Morgenroth, than by intraperitoneal injection. Bacteremia develops in addition to the local lesion, but it is less rapid, less severe and more closely duplicates the conditions of streptococcus infections of human beings. Pneumococcus infections are likewise produced by injecting the organisms directly into the lungs of mice, rats, guinea-pigs or rabbits and the resulting bacteremia is somewhat less severe. Anthrax bacilli may be injected subcutaneously rather than intraperitoneally, etc., the whole principle being to produce more or less localized lesions from which fatal systemic infections may spring as these are the usual events in bacterial diseases of human beings. It is true that in human beings some bacterial infections like typhoid fever, pneumonia, meningococcus meningitis, etc., may be bacteremias first with subsequent localizations; these conditions can hardly be duplicated in the lower animals but the final results are probably sufficiently well duplicated by producing local lesions first from which the blood becomes secondarily infected.

Chemotherapy of Experimental Localized Bacterial Infections of Serous Cavities.—Localized lesions of serous cavities also offer a special field of usefulness in chemotherapeutic research. Thus Sands and myself[15] have been able to produce well defined pneumococcus lesions by injecting intrapleurally 0.5 c.c. of twenty-four hour dextrose blood broth cultures of pneumococci of moderate virulence into the right pleural sacs of guinea pigs; as a general rule, this was followed by the development of acute suppurative plueritis on both sides with pericarditis. The lesions, however, were not strictly confined to the pleurae since both lungs frequently showed inflammatory changes and pneumococci were generally found in the blood. Extremely virulent cultures were unsatisfactory, inasmuch as rapidly fatal bacteremias ensued; cultures of moderate virulence in broth, rather than suspensions in saline solution, produced the best results, the broth probably aiding in the production of suppurative changes.

From our experiments it would appear that in experimental pneumococcus pleuritis sufficient ethylhydrocuprein hydrochlorid may be injected into the pleural sacs to render the contents decidedly pneumococcidal without

toxic effects, whereas the same amounts injected into the blood are without effect on the local lesions or pneumococcus bacteremia. Similar results have been observed by Idzumi and myself[16] in the treatment of experimental pneumococcus meningitis of rabbits with ethylhydrocuprein hydrochlorid.

In my opinion, the treatment of experimentally infected serous cavities and especially the pleural cavities, constitutes a procedure of distinct value in chemotherapeutic studies in bacterial infections when the curative dose of a compound is likely to be but slightly less than the maximum tolerated dose. In serum therapy or in chemotherapy with such arsenical compounds as arsphenamin in spirochetic or trypanosome (*Tr. equiperdum*) infections, when the curative amount is many times less than the toxic amount, generalized infections are suitable, since the compound may be given in relatively large doses without danger of poisoning.

Studies in the production of experimental tuberculous pleuritis by Ogawa and myself[17] have shown that it is possible to produce a tuberculous infection limited to the pleurae and lungs with such extension of the disease to the pericardium and lymph glands as occurs by direct lymphatic absorption and continuity of tissues. The intrapleural injection of guinea-pigs with bovine tubercle bacilli is followed by metastases to the spleen, liver, and other organs, that is, a generalized infection follows. Human tubercle bacilli likewise produce generalized infections but in not as high a percentage of animals. Human tubercle bacilli injected into the pleural cavities of adult rabbits generally (but not always) resulted in localized lesions; bovine bacilli regularly produced generalized lesions in these animals. Bovine and human tubercle bacilli injected into the pleural cavities of adult white rats and adult dogs have resulted in the production of localized lesions. By suspending the bacilli in broth instead of saline solution, the local suppurative changes generally have been increased. While extensive chemotherapeutic studies have not been made with these lesions, I believe that localized tuberculous pleuritis and pneumonitis offer more favorable lesions for studies *in vivo* than generalized and widespread lesions, the medicaments being injected into one or both pleural cavities at varying intervals after the injection of the bacilli into the right sac.

For the production of localized bacterial infections of serous cavities for purposes of chemotherapeutic research it would appear necessary to select test animals possessing some natural immunity for the test bacterium; furthermore, the bacterium should be of moderate rather than of extreme virulence. These factors decrease the chances of producing generalized infections and bacteremias and favor the development of localized lesions spreading only by direct continuity of tissues. Under these conditions subtoxic amounts of medicament injected into the cavities may produce therapeutic results even when diluted with inflammatory exudates and thereby yield information of value in chemotherapeutic investigations.

Value of Animal Experimentation in Relation to Bacterial Chemotherapy. This now logically brings us to the point where the advantages and limitations of chemotherapeutic investigations among the lower animals requires brief discussion. As stated in Chapter II, toxicity tests with new compounds are indispensable and especially when they are designed for administration by intravenous or intramuscular injection and even by ingestion; for purely local application to the skin or mucous membranes they may be dispensed with although even in this connection preliminary tests among the lower animals are advisable and especially when the medicament is to be used for instillation into the eye, urethra and bladder. Much too frequently new compounds are rushed into clinical use for the treatment of bacterial infec-

tions on the flimsiest sort of evidence of their probable value with few or no toxicity tests and with no data concerning their effects upon experimentally produced infections. It is true that antiseptics designed for local application cannot be easily tested on the lower animals, but all designed for the treatment of systemic infections should be first tested out by properly conducted animal tests.

Experiments of this kind will give at least some data bearing upon their effects in the treatment of an actual infection. When a new compound is found to be of relatively low toxicity and to possess an encouraging degree of bactericidal activity *in vitro* especially in a menstrum of serum or blood, it is a temptation to assume that it will prove efficacious *in vivo;* at least, it appears that such should be the case, but we know so little of the fate of chemical agents *in vivo* and of the fundamental laws of chemotherapy that we have no right to draw concusions or deductions until the compound has been actually tried out in experimental infections and this is usually possible. I do not mean to infer that a compound failing to favorably influence an experimental infection will prove worthless in the treatment of the corresponding disease of human beings, but experience has taught me that it is usually possible to make the animal test quite favorable for eliciting the possible curative properties of a chemical agent and that if it fails under these conditions, we should be extremely cautions in advising its use in the treatment of disease insofar as specific anti-bacterial properties are concerned, although it may possess certain pharmacological properties of general value aside from the purely chemotherapeutic applications.

The chief limitation of animal tests is the difficulty or impossibility of exactly duplicating the condition of the disease as it occurs in human beings and the tendency for making our tests unusually and unnecessarily severe. This is especially important when we remember that there is considerable data indicating that chemotherapy *in vivo* is probably not a mere matter of a disinfectant killing bacteria by direct action as it may in the test-tube but that certain synergistic tissue reactions may be required to bring about this result by more indirect means. Furthermore, the tissues of the lower animals may react in quite a different manner from those of human beings in this connection, as they are known to vary in reaction to some species of bacteria. The questions of chemistry of the blood and fixed tissues, natural immunity of the infecting organism and varying susceptibility to the toxic or organotropic effects of the compounds, are to be regarded as possible factors of variation and constituting limitations to the true value of animal tests for evaluating new compounds from the chemotherapeutic standpoint. But yet experience shows that, in a broad and general manner, animal tests may yield much more favorable results than observed in the treatment of human beings; at least, this is true in trypanosomiasis and syphilis and likewise in bacterial infections, as I shall discuss in Chapter VIII—devoted to the practical applications and results of chemotherapy in the bacterial diseases of human beings and the lower animals. Therefore, while it cannot be denied that compounds without demonstrable curative effects in experimental infections of the lower animals may be somewhat effective in human beings, yet it is a fact that the reverse of this is more common and indicating that we should always employ well conducted animal tests as part of our study of new chemical agents designed for the disinfection of tissues in systemic infections.

Various Methods for Chemotherapeutic Investigation in Experimental Bacterial and Mycotic Infections.—In this section I shall briefly summarize the more technical details for the production of various experimentally pro-

duced bacterial infections of the lower animals which have been found useful in chemotherapeutic investigations. It is obviously impossible to designate the exact amounts of organisms to employ as these vary greatly according to virulence, but the main principles of technic may be given. Needless to state all painful operations on the lower animals are to be conducted under anesthesia and with scrupulous care.

Methods for Chemotherapeutic Investigation in Experimental Tuberculosis.—Tuberculosis offers a particularly favorable infection for experimental chemotherapy because the duration of the disease may be prolonged over weeks or months without the production of acute overwhelming infections; this, however, may be also a disadvantage because of the long periods required for obtaining and evaluating the results of therapeutic experiments.

Local tuberculous lesions are readily produced in rabbits by inoculating the *cornea* or *anterior chamber of the eye* with virulent bacilli, preferably of the bovine type. My colleague, Dr. Harkins, has also produced *cutaneous* lesions by rubbing bovine bacilli into superficial abrasions of the skin of rabbits and by intradermal injections of very minute amounts of culture. After a few weeks, well-developed local lesions are produced which may be carefully watched and studied during treatment with drugs administered by intravenous or intraperitoneal injection or, in the case of the cutaneous lesions, by local application.

Ogawa and I[18] have also produced tuberculous *pleuritis* for the purpose of providing lesions for treatment with intrapleural and intravenous injections of drugs. In our work human and bovine strains of tubercle bacilli were inoculated into the right pleural cavities of rabbits, dogs, guinea-pigs and white rats. The distribution, type and severity of the experimental lesions varied considerably among the different species of animals and to some extent among the animals of the same species. As a general rule, the best developed lesions of the right pleura and lung were observed among animals having a certain degree of natural immunity to tubercle bacilli, such as the rabbit to human bacilli, the dog and rat to human and bovine bacilli.

Tuberculous *meningitis* may be produced by introducing the bacilli into the subarachanoid space. I have succeeded in infected dogs, rabbits and guinea-pigs by introducing the bacilli through a small trephine opening of the skull; successful inoculations have also been reported by intraspinal inoculations. In most of my experiments, however, distal lesions likewise developed in addition to progressive tuberculosis of the meninges.

Most experimental work in the chemotherapy of experimental tuberculosis has been conducted with *generalized infections* of guinea-pigs and calves inoculated by subcutaneous, intravenous or intraperitoneal injection of bovine bacilli. By careful selection of doses chronic and long continued, but progressive lesions may be produced admirably adapted for therapeutic tests.

The administration of drugs may begin any time after inoculation. Possibly the most favorable results are to be obtained by giving the first dose one or two days after inoculation to be followed by subsequent doses at chosen intervals, or the lesions may be allowed to develop before treatment is begun and thereby duplicate more closely the problems arising on the chemotherapy of tuberculosis of human beings involving the very important question of the penetrability of the drug for tubercles in various stages of formation and necrosis. For routine work guinea pigs inoculated with 0.1 to 0.01 mg. of bovine bacilli by subcutaneous injection may be used and treatment begun with two or more animals next day, with several others about three days later, others one week later, etc. The drug may be given by intravenous, intraperitoneal or subcutaneous injection at chosen intervals and in every experiment several untreated controls are to be included. The results are evaluated according to the duration of life and the progress of lesions, or necropsies may be conducted at a chosen time, as six weeks after inoculation, and the distribution and size of the lesions compared with those in the untreated controls.

Methods for Chemotherapeutic Investigation in Experimental Pneumococcus Infections.—Most investigation in the chemotherapy of pneumococcus infections have been conducted with mice according to the method in general use for determining the protective and curative activity of antipneumococcus serum.

In this method, *systemic or generalized infections* are produced by intraperitoneal or subcutaneous injection of mice with virulent pneumococci, the dose being based upon a preliminary virulence test. It is necessary to avoid overwhelming infections, the aim being to inoculate with a dose producing fatal bacteremia in four or five days; larger doses proving fatal in forty-eight hours are too severe and smaller doses may yield irregular results. My usual plan has been to determine the smallest lethal dose of an eighteen-hour blood dextrose broth culture in a period of five days and to inoculate for the main test with 10 to 50 times this amount. The drug may be injected intravenously, intraperitoneally or subcutaneously to some mice at the time of infection; others may be treated

two, four and twenty-four hours after inoculation. In each test several inoculated but untreated mice are to be included as well as two or more receiving the drug alone (controls). The results are usually evaluated according to the duration of life as compared with the untreated controls; a better way however, is to treat a series of mice with the same dose of compound and to express the results according to the percentage of survivals.

A second method, and one which I believe is to be preferred in some respects for the production of systemic infections, consists in giving a series of mice a daily subcutaneous injection of culture equal to the minimal fatal dose by intraperitoneal inoculation. The drug is also given daily by intraperitoneal injection 4 hours after each inoculation. Controls receive the culture alone and the drug alone in the usual manner. By this method acute overwhelming infections are avoided although fatal bacteremias are gradually produced in the untreated animals. If the controls do not show signs of infection by the third day the dose of culture may be increased at will and the experiment terminated when the untreated animals succumb; the results are expressed according to the smallest curative dose of compound per kilogram of weight.

These tests with mice may be modified by injecting the pneumococci directly into the lungs through the chest walls; small areas of pneumonia may be produced with bacteremia.

Rabbits may be employed instead of mice in the same kind of tests; guinea pigs and white rats, however, are more refractory, although I have used the latter as substitutes for mice with success by using highly virulent cultures.

Experimental *pneumonias* may be produced in dogs by introducing large amounts of culture directly into the trachea after the method of Wollstein and Meltzer;[19] rabbits may be used in the same manner according to the method described by Winternitz and Hirschfelder[20] or the culture may be injected directly into the trachea with a syringe or through a catheter introduced into the trachea by means of a small incision below the larynx. Monkeys, however, are probably best adapted for the production of pneumonias according to the methods developed by Blake and Cecil.[21]

Idzumi,[22] working in my laboratory, has produced *meningitis* in rabbits and dogs. The subthecal injection of virulent pneumococci into the lumbar region of the spinal cord of rabbits was usually followed by the development of a fatal suppurative leptomeningitis accompanied by definite changes in the tissues and spinal fluid, fever, leukocytosis and clinical symptoms of meningitis, such as hyperesthesia, opisthotonos and convulsions. These changes appeared to be sufficiently definite to permit the use of large rabbits for chemotherapeutic experiments in pneumococcus meningitis when they are infected by the direct subthecal injection of virulent pnuemococci. Meningitis may be produced in dogs by the subthecal injection of large amounts of highly virulent pneumococci; injections into the cisterna magna yield better lesions and have proven extremely valuable for chemotherapeutic studies in meningitis.

Sands and I[23] have also produced an experimental *pleuritis* in guinea-pigs by the injection of 1 c.c. of a twenty-four hour dextrose blood broth culture of virulent pneumococci into the right pleural cavity of guinea-pigs; acute suppurative pleuritis on both sides associated with suppurative paricarditis followed, which generally terminated fatally in three or four days with bacteremia. Chemotherapeutic experiments were conducted with optochin by direct intrapleural injection and offered new opportunities for the chemotherapeutic investigation of localized infections.

Methods for Chemotherapeutic Investigations in Experimental Streptococcus Infections.—Similar methods may be employed with streptococci due care being taken to work with a strain of high virulence maintained by occasional animal passage.

For *systemic infections* mice are well adapted for routine tests and especially when inoculated by injection under the skin of the thorax according to Morgenroth's method. Within forty-eight hours severe local lesions are produced characterized by extensive edema and containing myriads of organisms; the blood is gradually infected and streptococci are almost invariably found in the heart when cultured at necropsy. Preliminary virulence tests are required for determining the proper dose of culture to inject, the object being to use in the main tests a single dose capable of producing this lesion and proving fatal in about five to seven days.

Mice are now inoculated with this amount of culture. The drug may be given by intraperitoneal injection; sometimes the intravenous or subcutaneous routes are to be preferred. As a rule, I give the first dose of drug four hours after inoculation but one may lengthen this interval to twenty-four hours or longer when the local lesions are well under way. Subsequent doses are given at daily intervals. Untreated controls and controls receiving the drug alone are to be included in each experiment and the results evaluated according to the effects upon the local lesion, the duration of life and the cultures of the blood, as compared with the inoculated but untreated controls.

A modification of this test consists in giving the mice a daily injection of culture in about one tenth the single fatal dose. The drug is injected four hours after each inoculation and the injections continued until the inoculated but untreated controls have

succumbed, when the results are evaluated according to the size of the local lesions and duration of life among the treated animals. If one-tenth the minimal fatal dose fails to produce local lesions in the controls by the third or fourth day, the dose of culture should be raised. By this technic, it is sometimes possible to continue the experiment for ten days or longer and the milder, but continuous infection more closely resembles the conditions of streptococcus infections among human beings and affords a more favorable lesion for eliciting the possible curative effects of compounds than the acute overwhelming infections following inoculation with single large doses of culture.

Rabbits may be employed in much the same manner and are particularly serviceable when it is desired to give repeated doses of the drug by intravenous injection. The method of daily subcutaneous injections of streptococci followed two to six hours later by the administration of the drug is a particularly good one and the dose of culture may be increased or decreased according to the condition of the untreated controls.

Rats and guinea-pigs are much more resistant to streptococci but may be employed if a highly virulent culture is available; I have repeatedly used the white rat with satisfactory results.

Streptococcus *pneumonia* and *empyema* has been produced in dogs by Wollstein and Meltzer,[24] and likewise by Graham and Bell,[25] by means of intrabronchial insufflation of cultures or empyema fluid. Gay and Rhodes[26] have employed rabbits infected by intrabronchial insufflation with 1 to 10 c.c. of a passage culture of hemolytic streptococcus. Histologically, this pneumonia was lobular in distribution, necrotizing in effect, did not resolve readily, and was characterized by peribronchial and perivascular edema and later infiltration of mononuclear cells (interstitial bronchopneumonia). It was quite different in character from the pneumonia produced by the pneumococcus. These differences were further marked by the occurrence of pleurisy with effusion, involving by extension both pleural cavities and the pericardium. Bacteremia occurred only irregularly.

Within the past year, Miss Rule and I have produced streptococcus *meningitis* for chemotherapeutic studies in this disease with a virulent hemolytic strain introduced into the subarachanoid space. Rabbits were found unsuitable because of early death due to bacteremia. Rats and guinea-pigs being more resistant were found more satisfactory and especially white rats of 200 to 300 grains inoculated through a small trephine opening near the middle line of the calvarium. Cats and especially dogs are also suitable and these may be infected by intralumbar or intracisternal injection. The dose of culture to employ must be determined in preliminary tests and dilutions may be made with sterile broth instead of saline solution as this aids in producing a more extensive exudation of leucocytes. Drugs are given by intravenous or intracisternal injection or through the same trephine opening employed for inoculation. It is a comparatively easy matter to produce acute meningitis by these means with purulent cerebrospinal fluids containing enormous numbers of streptococci.

Methods for Chemotherapeutic Investigation in Experimental Staphylococcus Infections.—Since most of the lower animals possess a high natural immunity to staphylococci it is more difficult to conduct chemotherapeutic investigations with these organisms. Rabbits, however, may be injected intravenously with *Staphylococcus aureus* in sufficient amounts to produce abscesses in the kidneys, heart, diaphragm, etc. As a general rule, preliminary tests are required for the purpose of determining how much of a twenty-four hour broth culture injected intravenously is required for producing these lesions of the internal organs. In the main tests each animal may be given this dose of culture to be followed four hours later by the first dose of drug with daily injections thereafter for about a week or ten days, when all surviving animals should be necropsied and the extent of involvement among the treated animals compared with the untreated controls. A better method, however, is to give a daily injection of culture in smaller dose followed by a daily dose of drug four to six hours after each inoculation. Controls receiving the cocci alone and drug alone should be included. By gradually raising the dose of culture, it is possible to produce fatal infections in the untreated controls within a week to ten days, but as a general rule it is required to terminate the experiment arbitrarily at about this time and evaluate the results on the basis of macroscopic and microscopic examination of the kidneys, heart and other organs of the untreated controls as compared with the treated animals.

I have also produced satisfactory lesions in the white rat by injecting virulent staphylocci intravenously in a single large dose found sufficient in preliminary tests for producing abscesses in the kidneys as determined by necropsy examination five to seven days later. The drug may be given intravenously or intraperitoneally either in a single large or multiple small doses, the first dose being given in two to twenty four hours after inoculation and before the infection has made too much headway. Cultures of the heart blood in both rabbits and rats have sometimes showed that bacteremia may develop but the animals do not succumb as rapidly or as regularly as with pneumococcus and streptococcus infections.

Virulent staphylococci may be injected subcutaneously in rabbits with the production of local abscesses, but in my experience bacteremias and abscesses of the kidneys and other internal organs have developed only exceptionally and irregularly.

Methods for Chemotherapeutic Investigations in Experimental Anthrax Infections.—White mice and rabbits are commonly employed for chemotherapeutic studies in anthrax; both animals, however, are very susceptible and if a highly virulent culture is employed amounts almost unbelievably small may suffice for producing rapidly fatal bacteremias. My colleague, Dr. Harkins, has observed that mice inoculated subcutaneously with a dose so small that cultures prove sterile, like 0.00000001 c.c., may produce a fatal infection; in other words this may mean a single bacillus or spore unable to proliferate in an artificial culture medium but yet capable of rapidly infecting a mouse. It is necessary, therefore, to first determine the virulence of a culture for mice or rabbits by injecting various amounts subcutaneously and choosing for the main tests an amount about ten times higher than the minimal fatal dose at the end of five to seven days. Subcutaneous edema usually follows and at death anthrax bacilli are to be found in the blood of the heart by smear and culture and in the liver, spleen and other organs by histological examination.

The drug may be given intraperitoneally to mice and intravenously to rabbits. It is generally advisable to give the first dose in two to four hours after inoculation and to follow this by daily injections. The usual culture and drug controls should be included and the results evaluated according to the duration of life or percentage of survivals among the treated animals.

With rabbits it is possible to adopt the method of giving a daily subcutaneous injection of culture followed in four hours by a daily injection of the drug. In this manner milder and more prolonged infections may be produced somewhat more favorable for eliciting the possible curative properties of the drug under study.

Methods for Chemotherapeutic Investigations in Experimental B. Coli and Typhoid Infections.—The experimental production of *B. coli* and *B. typhosus* infections among the lower animals have not been very successful. It is true that the daily intravenous inoculation of rabbits with *B. coli* or *B. typhosus* may produce fever and wasting with cloudy swelling of the kidneys, but local lesions comparable to those occurring among human beings are not produced, the effects being due to the toxins of the organisms. *B. coli* infections are of interest mainly from the standpoint of the experimental production pyelitis and cystitis and the possibility of influencing these therapeutically by the administration of such compounds a hexamethylamin and hexy-resorcinal.

I have been occasionally successful in infecting the bladders of female rabbits by repeated direct inoculation with cultures of *B. coli* freshly isolated from human infections, but in no instance so far has a pyelitis followed and the cystitis has tended to heal and disappear spontaneously. It is possible, however, to experiment along these lines in conjunction with tests for an increased bactericidal activity of the urine as previously described. By infecting the bladder with the introduction of 1 or 2 c.c. of a twenty-four-hour broth culture followed in about four hours by the administration of the drug and 30 to 50 c.c. of water by the stomach tube, it is possible to study the bactericidal effects of the drug in the bladder by removing specimens of urine as aseptically as possible at subsequent intervals. In my experience female rabbits are to be preferred but male rabbits may be employed although it is extremely difficult to secure urine without contamination.

A complete review of attempts to produce experimental typhoid infections among the lower animals has been given by Gay;[27] with the exception of a few successful results with apes these experiments have usually failed to reproduce the lesions commonly found in human beings. Gay and Claypoole,[28] however, believe that it is possible to produce a carrier state in rabbits which may prove useful for chemotherapeutic investigations.

Methods for Chemotherapeutic Investigations in Experimental Mycotic Infections.—It is possible to transmit some of the mycotic infections and especially the ring-worms to the lower animals and produce lesions well adapted for chemotherapeutic experiments by the local application or systemic administration of drugs. Young guinea-pigs are especially suitable, the abdomen being shaved and the organisms inoculated into superficial abrasions in two different areas, one area being reserved for control purposes when cutaneous applications of compounds are made. The shaven ears of rabbits may be likewise employed.

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CHAPTER V

ANILIN DYES IN THE CHEMOTHERAPY OF BACTERIAL DISEASES

As stated in Chapter III in the historical review of chemotherapeutic investigations in bacterial diseases, the anilin dyes have long held a place of primary interest and importance, not only from the standpoint of displaying a direct bactericidal activity themselves, but also as possible carriers of more potent substances to the protoplasm of the organisms. While we now know that mere staining of bacteria does not necessarily indicate their death, yet the idea and hope of utilizing dyestuffs for the disinfection of the tissues have been long entertained and interest in the subject greatly renewed within recent years. Naturally a very extensive literature has accumulated upon the subject and especially upon the bacteriostatic and bactericidal activity of the dyes *in vitro*, but unfortunately most of these reports can be reviewed only in a broad and general manner, because the methods employed have varied greatly and the influence of technic upon the results is so tremendous that fine distinctions and deductions cannot be drawn.

Classification of Dyes in Relation to Chemotherapy.—Practically only the synthetic dyestuffs prepared from coal tar have been employed for the staining of bacteria and for bacteriostatic and bactericidal purposes. Natural dyes, like Indian-yellow, quercetin, chrysin, cochineal, carmine, cudbear, hydrastis, etc., have been proven to be without parasiticidal properties and have not been employed for these purposes.

A very large number of dyes have been prepared from coal tar and their chemistry is exceedingly involved, the exact constitution of not a few being still in dispute and their preparation kept as trade secrets. Furthermore, the various dyes available are not always in a pure state and sometimes vary greatly in their staining properties, toxicity, bacteriostatic and bactericidal activity.

The large number of organic dyestuffs which have been synthetically prepared show that the dyeing property is dependent upon structure and a

theoretical classification has been based according to the chromophore group occurring in them; a very useful description of the chemistry of those employed in the textile industries is given by Cain and Thorpe.[1] The following classification is after that given by them and in each group I have included some of the dyes which have been employed in bacteriological work and to a more or less extent in experimental chemotherapeutic investigations:

(1) The *nitroso-dyes*, or nitroso-phenols produced by the action of nitrous acid on the phenols, and embracing such dyes as fast or solid green, naphthol-green B, gambine Y, etc.

(2) The *nitro-dyes*, produced by the introduction of the nitro group into the molecule of the aromatic phenols, and including picric acid, martius-yellow, naphthol-yellow S, etc.

(3) The *azo-dyes*, constituting an important group including such well known dyes as trypan-blue and trypan-red, prepared by the interaction of a diazo-salt with an aromatic hydroxy-compound or amin, and embracing such amidoazo dyes as acid-yellow, chrysoidin, sulphon acid-blue R; oxyazo-dyes, as ponceau-fast red, buffalo-fast crimson R, Columbia-blue R and a host of others; diazo-dyes as benzo-azurin, palatine black, resorcin-brown, sudan III, diamond-green, brilliant crocein M, etc. Belonging to this same group are also the dyes from tetrazo-salts like Congo-red, benzopurpurin B, diamin fast-red F, etc.; such triazo-dyes as Columbia-green A, benzo-fast blue B, direct black V, and such tetrakisazo-dyes as benzo-brown G, hessian brown BB, dianil-black PR, etc.

(4) The *triphenylmethane dyes*, probably the most important group of all from the standpoint of chemotherapy, generally considered as anhydro derivatives of triphenyl-carbinal, and including such dyes as basic fuchsin, acid fuchsin, parafuchsin, gentian-violet, magenta, hexamethyl-violet, hexaethyl-violet, methyl-green, Victoria-blue, nightblue, Dobner's violet, malachite green, brilliant green, acid-green, glacier-blue, setoglaucin, setocyanin, water-blue B, new fast green, etc.

(5) The *diphenylamin dyes* also include several of special interest from the standpoint of chemotherapy, embracing the indophenols, thiazins, oxazines, indamines and azures and including such dyes as methylene-blue, thiopyronin, toluylene-red, safranin, Bundschedler-green, nile blue By, neutral red, etc.

(6) The *acridin dyes*, which are amido derivatives of phenylacridin and of acridin base and including acriflavin, proflavin and a new compound 2-aethoxy-6, 9-diamino-acridin-chlorhydrate prepared by Morgenroth, Schnitzer and Rosenberg[2] and known under the trade name of "rivanol." Acriflavin is the name given to "trypaflavin" by the Medical Research Committee of Great Britain because the latter was a registered trade mark; the compound was first prepared by Benda[3] at Ehrlich's instigation in 1911, and was found to have such a marked therapeutic effect in trypanosomiasis that the name "trypa-flavin" was given to it. Proflavin is another acridin derivative, diaminoacridin, and appears to be easier and cheaper to manufacture and about equal in bactericidal activity but of lesser toxicity. Probably acriflavin has commanded more attention than any other dye and especially since it is apparently non-injurious to the tissues, diffuses readily in them and possesses other properties of value in the treatment of wounds. It may not be amiss to state here that the names of various other compounds ending in "flavin" are of commercial appellation and have no scientific generic basis, the "flavins" being yellow dyes. Acriflavin connotes a yellow derivative of acridin; thioflavin means a yellow substance containing sulphur. Other "flavins" have names coined in the same manner.

(7) The *pyronin dyes* including both diphenylmethane and triphenylmethane derivatives, like pyronin G, acridin-red, fluorescein and its sodium salt (uranin), fast acid-violets, fast acid-blue, the eosins, etc.

(8) Dyes of the *auramin* series, quinolins like cyanin B and indigoid colors like scarlet 6R, have been much less frequently employed.

Bacteriostatic and Bactericidal Activity of the Synthetic Dyes in Relation to Chemotherapy.—The synthetic dyes vary greatly in bacteriostatic and bactericidal activity. Some dyes, and especially certain members of the triphenylmethane and acridin series, like gentian-violet, acid and basic fuchsin, brilliant green, acriflavin, proflavin and rivanol, are very highly bactericidal, while others like methylene-blue and fluorescein are relatively feeble and mainly employed in chemotherapy for the purpose of combination with mercury or some other more highly bactericidal substances for aiding penetrability. Gentian-violet and magenta may inhibit the growth of staphylococci and streptococci in culture media in dilutions as high as 1:1,000,000 and 1:100,000 dilutions in agar or egg medium and are commonly

employed for aiding in the isolation of tubercle bacilli and organisms of the typhoid-colon group.

As a general rule, however, the majority of anilin dyes are relatively feeble in bactericidal activity and especially in the presence of serum, blood, pus and other organic matters. Probably gentian-violet, acid fuchsin, brilliant green and acriflavin possess the highest bacteriostatic and bactericidal properties, gentian-violet being particularly destructive for Gram-positive bacteria and the fuchsins for Gram-negative bacilli. Even dyes of the same series vary greatly in these properties, to which further reference will be made shortly when discussing selective activities; here it may be stated that while many of these dyes are sufficiently bacteriostatic and bactericidal *in vitro* to be of great aid in the isolation of some organisms, some are relatively feeble in the chemical disinfection of the tissues by local or topical application or by intravenous injection.

Bactericidal Activity of the Dyes in Vitro.—Gay and Morrison,[4] in a recent study of the bactericidal properties of a large number of dyes, have observed that in a general way both streptococcus and staphylococcus are more susceptible to dye-stuffs, with few exceptions, than is *B. typhosus*. Generally speaking, the streptococcus studied was more susceptible than the strain of staphylococcus. It would seem from their experiments that diazo dyes have relatively little bactericidal properties and that the most active dyes for streptococcus belonged to the triphenylmethane and acridin groups.

My own observations, however, have not shown as high bactericidal effects on the part of a number of anilin dyes for these three organisms as observed by Gay and Morrison. The technic employed, however, was radically different since the exposure allowed for the action of the dyes on the organisms was only five minutes and affords additional proof of the statement that the dyes act rather slowly. For example, gentian-violet in saline solution was completely bactericidal for a streptococcus in only 1:1000 to 1:1500 in this exposure, while for a culture of *Staphylococcus aureus* the bactericidal dilution varied from 1:2000 to 1:2400. Krigler[5] has shown the marked influence of the composition and reaction of the medium upon the behavior of the dyes *in vitro* and has employed a peptone medium with a pH of 7.1 in testing a large number of organic substances including many of the dyes, against various Gram-negative and -positive organisms. Norton and Davis[6] have also used a number of the dyes incorporated in culture media in attempts to work out a method for differentiating *streptococcus viridans* from pneumococci, but without success. Acid dyes or salts of acid or basic dyes were found to be practically inert; in their opinion a dye to possess bacteriostatic action must contain three benzol rings and two or more amino-groups in which the hydrogen atoms have been substituted by alkyl radicles.

The dyes have also been studied in relation to bactericidal activity *in vitro* for the *tubercle bacillus*, some of the earliest chemotherapeutic experiments in tuberculosis being made with these substances. Lewis,[7] with the assistance of Kraus, found that many of the triphenylmethane dyes as a group were more bacteriostatic and bactericidal for the pneumococcus and *Staphylococcus aureus* than for the tubercle bacillus, while the azo-dyes as a group inhibited the growth of tubercle bacilli more readily than these other organisms. Unfortunately, however, trypan-red and trypan-blue, which are capable of penetrating to the centers of fully formed tubercles in rabbits and guinea-pigs within a few hours after injection, have been found by DeWitt[8] and others almost without bactericidal power for tubercle bacilli both in the test tube and in infected animals. Lewis, DeWitt and others have prepared many new compounds of trypan-red and trypan-blue with iodin, phenol and the metals and while bactericidal activities *in vitro* were sometimes increased, unfortunately penetrability for tubercles was usually lost, so that the results of treatment of infected animals were largely negative. The same was largely true of new compounds of Niagra-blue with iodin, phenol and certain fatty acids. One compound of the dye with formic acid showed a great increase of bacteriostatic power *in vitro* without loss of power to stain the tuberculous tissues. Of several diazo-creosote combinations, one violet blue in color, inhibited the growth of tubercle bacilli in dilution as high as 1:100,000 and appeared to extend the lives of a few treated animals.

Because of the unusual fat content of tubercle bacilli, it was early thought that fat soluble and fat staining dyes might have a special action on tubercle bacilli, but Corper[9] has found no inhibitory action of fat dyes on the growth of tubercle bacilli, no power to penetrate living tubercles and no therapeutic effect in experimental tuberculosis of guinea-pigs.

Probably methylene-blue and its various combinations have been more extensively studied in tuberculosis than any other dye. DeWitt[10] has found that 1:10,000 dilutions

may prove bacteriostatic *in vitro* and bactericidal in some cases in 1:100 solutions though weaker solutions were not tried. Animals inoculated with bacilli previously exposed to 1:100 solutions usually developed a milder infection with a longer period of incubation, but the dye and a large number of combinations, yielded almost negative results in the treatment of tuberculous guinea-pigs, which subject I will review shortly in a more detailed discussion of the action of the dyes *in vivo*.

Of great importance in relation to the chemotherapy of infected wounds and various localized infections of the skin, mucous membranes and serous cavities, is the question of bactericidal activity in the presence of blood, serum and pus. Browning and Gulbranson[11] in 1917 discovered that the bactericidal activity of acriflavin is apparently enhanced by admixture with serum, though greatly diminished by pus. Hewlett,[12] Dakin and Dunham,[13] Drummond and McKee[14] and others have observed essentially similar results in serum and defibrinated blood, not only with acriflavin, but with proflavin as well; others, however, like Morgan,[15] Mueller[16] and Graham-Smith[17] have not arrived at similar conclusions. Gay and Morrison, working with a pus produced experimentally by the intrapleural injection of rabbits with virulent streptococci, found that most of the dyes studied lost markedly in bactericidal power in the presence of pus. A considerable number remained rather active and a few of them like safranin, methylene-green, basic fuchsin, rhodulin-violet and two flavin compounds were as active in pus as in broth; acriflavin was actually more active in pus than in broth.

Curiously the bactericidal activity of gentian-violet is reduced much more markedly by blood than by serum. I have observed, for example, that in a mixture of 4 c.c. of broth plus 0.5 c.c. of a culture of *streptococcus* and 0.5 c.c. of 1:100 gentian-violet (final concentration 1:1000), that complete sterilization occurs in a minute or less; similar results occurred when sterile serum was used instead of broth, but when whole citrated human blood was employed, complete sterilization did not occur in even an hour. Identical results were observed with a culture of *Staphylococcus aureus*.

From the standpoint of the treatment of infected wounds and various infections of the skin, mucous membranes and serous cavities or of systemic infections by intravenous injection, the anilin dyes are relatively feeble and their choice narrowed down largely to acriflavin, proflavin, gentian-violet, acid and basic fuchsin and possibly malachite green. This subject however, will shortly receive further consideration from the standpoint of their value in the actual treatment of localized and systemic infections.

Bactericidal Activity of the Dyes in the Blood and Urine in Vivo.—Of great interest and importance in relation to the chemotherapy of bacteremia is the question of bactericidal activity and fate of the dyes when introduced into the blood, usually by intravenous injection. This has been studied by injecting the dyes and withdrawing blood at intervals for bactericidal tests and also by producing experimental bacteremias of rabbits and treating with intravenous injections of the dyes. In 1913, Churchman and Herz[18] reported that the intravenous injection of a rabbit with 10 c.c. of a 1:200 solution of gentian-violet (0.05 gm.), corresponding roughly to the rather large dose of approximately 0.025 gm. per kilogram of weight, was followed by a loss of selective bactericidal activity of the blood about one hour later. Evidently this is due to its removal from the blood by the fixed tissues since further experiments consisting in the addition of the dye to blood *in vitro* showed no loss or reduction in selective bactericidal effects. Churchman[19] also states that he has not been able to kill organisms circulating in the blood by intravascular injections of gentian-violet, but I will later refer to this subject in a discussion of the use of the dyes in the treatment of experimental bacterial infections. Browning and Gulbranson state that intravenous injections of acriflavin temporarily increase the bactericidal effects of the blood, but I have found that these effects for streptococci disappear within an hour after the injection of rabbits with 0.010 gm. per kilogram of weight.

It would appear, therefore, that large doses of gentian-violet and acriflavin injected intravenously may at least temporarily increase the bactericidal activity of the blood (for about an hour), but these effects may not be sufficient for materially influencing a severe experimentally produced bacteremia since the dyes are rapidly absorbed by the tissues but, the bactericidal effects may be better in the less severe bacteremias of human beings.

Of further interest in this connection, and especially in relation to the treatment of bacterial infections of the kidneys and bladder, are the observations of Young and Hill[20] who found that the urine of rabbits removed aseptically by catherization following the intravenous injection of 0.005 to 0.010 gm. of gentian-violet per kilogram of weight was bacteriostatic and bactericidal for staphylococci and the colon bacillus.

Bactericidal Activity of Mixtures of the Dyes.—The question of the bactericidal activity of mixtures of various dyes deserves brief consideration. Gay and Morrison have tested various combinations for streptococci, staphylococci and typhoid bacilli and found that the final bactericidal dilution of such mixtures might reach the limit of the stronger dye, but in case of interaction and precipitation between the two solutions the bactericidal effect was actually decreased. Churchman[21] has observed that mixtures of gentian-violet and neutral acriflavin are bactericidal; also mixtures of gentian-violet and fuchsin, the gentian-violet for Gram-positive organisms and the acriflavin and fuchsin for Gram-negative organisms. In my experiments these observations have been confirmed and in the mixtures the identity of the dyes appear to be well preserved in that the bactericidal effects of the mixture for Gram-positive organisms is approximately the same as shown by the gentian-violet alone in the same strength and the bactericidal effect for Gram-negative organisms being about the same as shown by the acriflavin or fuchsin alone in the same strength. Churchman has advocated, therefore, the use of mixtures of these kinds for the disinfection of tissues infected with both Gram-positive and -negative organisms, to which further reference will be made in a succeeding chapter.

Influence of Heat upon the Bactericidal Activity of the Anilin Dyes.—Since heat tends to increase the rapidity and rate of chemical reactions, it is to be expected that hot solutions of the anilin dyes will prove more bacteriostatic and bactericidal than cold solutions and Churchman[22] has furnished definite proof that moderate heating (55°C. for 1 hour or 50°C. for 1½ hours) increases the penetrative power and bactericidal activity of gentian-violet. Working with an impeccable gentian-violet-fast strain, he found gentian-violet without effect at 22° to 35°C., but bactericidal when the solution of dye was heated to 50°C.; control tests showed that the effects were not due to heat alone or the dye alone, but to the combination of heat plus dye. The phenomenon has not been explained but the facts established indicate that when the dyes are used for the treatment of localized infections by direct application, the solutions should be comfortably hot in order to secure the enhanced bactericidal effects.

Selective Bacteriostatic and Bactericidal Activity of the Anilin Dyes in Relation to Chemotherapy.—As would be expected, the anilin dyes vary greatly in bactericidal activity and, as shown above, may exert a stronger bacteriostatic and bactericidal activity for some organisms than for others. Most of the anilin dyes in high concentration, say 1:100, will kill practically all organisms irrespective of whether they are cocci or bacilli, Gram-positive or Gram-negative, spore formers or non-spore formers, virulent or non-virulent, but some of them show bactericidal and bacteriostatic properties for some organisms in concentrations without effect upon other organisms. This is designated as "selective activity" and bears a very intimate relationship to chemotherapy, since it appears that the chemical constitution of the molecule influences these specific properties.

This selective bacteriostatic and bactericidal activity of the anilin dyes, first pointed out by Rozsahegyi[23] in 1887, the literature being fully described by Simon and Wood,[24] has long been utilized for aiding in the isolation of bacteria in cultures, as first shown by Drigalski and Conradi in their well-known medium employing crystal-violet for the isolation of typhoid bacilli from the feces. Since then a large number of special media have been

advocated on the principle of incorporating an anilin dye for the purpose of restraining certain bacteria while allowing others to grow.

Churchman[25] has especially contributed a most valuable series of studies bearing upon this important topic of selective activity of the dyes. He has clearly proved that gentian-violet, magenta and other dyes of the triphenylmethane series in general, possess a selective bacteriostatic and bactericidal activity for the Gram-positive organisms and especially the spore formers, arbitrarily designated as "normal activity;" curiously enough, however, these dyes may not inhibit the growth of the Gram-positive tubercle bacillus, a fact utilized by Petroff in the preparation of his valuable egg medium for the isolation of these organisms. While Churchman originally believed that the bacteriostatic activity of gentian-violet closely followed the Gram reaction, that is, was effective against Gram-positive organisms but not against *B. coli* and other Gram-negative bacilli, he has more recently[26] discovered that the process is not so specific, since he found in a culture of the colon bacillus two different strains, one which was restrained by gentian-violet and one which was not; Churchman has designated this as "microbic dissociation" and the discovery indicates how involved and complicated is this subject of the selective activity of the dyes.

Furthermore, dyes belonging to the same series may show opposite effects. For example, acid and basic fuchsins also belong to the triphenylmethane dyes, but yet, as shown by Churchman,[27] these restrain and kill Gram-negative organisms like *B. coli*, *B. pyocyaneus*, etc., designated as "reverse selective activity," whereas gentian-violet kills the Gram-positives like *B. diphtheriae*, *B. anthracis*, the staphylococci, streptococci, etc. In other words, such closely related dyes as basic triphenylmethanes (magenta) differ in bacteriostatic power from acid triphenylmethanes (the sulphonated compound acid fuchsin), since the former is more active than the latter. I will shortly discuss the matter of chemical constitution in relation to this phenomenon, but these discoveries by Churchman have shown to what extremes the selective activity of the anilin dyes may reach. This "reverse selective activity" of the anilin dyes has enabled Churchman[28] to isolate organisms in pure culture, as mixtures of *B. pyocyaneus* (Gram-negative) and *B. megatherium* (Gram-positive), by the use of these two dyes.

But the phenomenon of "reverse selective activity" on the part of some of the anilin dyes is also influenced by the medium in which they are incorporated. I have already discussed above the observation that the bactericidal activity of practically all of these dyes is reduced in the presence of serum, pus and blood, the acridin dyes like acriflavin, proflavin and rivanol being noteworthy exceptions; but Churchman[29] has shown that, while gentian-violet inhibits the Gram-positives and spares the Gram-negatives, acid fuchsin, related sulfonic substances, acriflavin and proflavin act in a different manner. When these are added to the agar (extrinsic bacteriostatis), the Gram-positive spore bearers are inhibited and the Gram-negatives unaffected; when added directly to the bacteria (intrinsic bacteriostasis) followed by transplanting the stained bacteria to agar, exactly opposite effects are noted since the Gram-negatives were inhibited and the Gram-positive spore bearers unaffected.

Churchman[30] has also shown that gentian-violet possesses a selective activity in the staining of bacteria and tissue cells, since the dye may penetrate and stain the nuclei of cells without destroying them while Gram-positive bacteria are killed; he has utilized this discovery in the treatment of arthritis and other localized infections. The use of brilliant green for the destruction of *B. diphtheriae* in carriers, of acriflavin for the removal of menin-

gococci in carriers, of various dyes in the treatment of urethritis, conjunctivitis, etc., are based upon this difference in toxicity for tissue cells and organisms. But in the opinion of Burke and Skinner[31] the selective bactericidal action of the triphenylmethane dyes applies only to the vegetative forms since the spores are highly resistant.

The list of examples of selective bacteriostasis and bactericidal activities of these dyes by Shiga,[32] Browning and Gilmour[33] and others may be extended to greater length, but without particular profit, since we are here mainly interested in the principles involved in relation to chemotherapy. But the subject is of great importance in relation to the chemotherapy of bacterial infections, since knowledge already gained indicates that when a dye is to be used for the treatment of a localized infection, it should be selected on the basis of greatest bacteriostatic and bactericidal activity in pus for the particular kind of organism present; at least to the extent of selecting gentian-violet for Gram-positive and acid fuchsin or acriflavin for Gram-negative bacteria. Furthermore, if new compounds of the dyes are to be synthesized, like compounds with mercury, copper, silver, etc., the selection of the dye should be influenced according to the particular group of organisms for which the compound is intended in order that the bactericidal activity of the compound may be enhanced by the intrinsic bactericidal activity of the dye. It is possible that mercurochrome prepared with a triphenylmethane dye, instead of fluorescein, may be improved upon in this manner, although I hasten to add that the subject is by no means as simple as it reads since the chemical manipulations may greatly reduce the bactericidal activity of the dye, as I well know from experience in the synthesizing of compounds of the anilin dyes with the more highly bactericidal heavy metals.

Chemistry of the Anilin Dyes in Relation to Chemotherapy.—Unfortunately, but little may be written at the present time upon this very important subject of the relation of chemical constitution of the anilin dyes to their bacteriostatic and bactericidal activities. Experts in the dyeing industries have worked out certain laws governing the dyeing of fabrics in relation to chemical constitution of both dye and fabric, but our information in relation to the bacteria is very meagre. It is practically certain, however, as shown by Churchman and others, that staining or dyeing of living bacterial cells does not necessarily kill them and that the bactericidal portion of a dye may be indeed a colorless portion, these observations indicating that the relation between chemical constitution and disinfection is much more complicated than between chemical constitution and mere protoplasmic staining.

But some information has been gained in this important subject. Browning and Gilmour,[34] for example, working with the *triamidotriphenylmethane* group of dyes observed that the unsubstituted triphenylmethane compound (pararosanilin) has only a comparatively weak bactericidal action. The substitution of alkyl-groups in the amido-side-chains (hexamethyl- and hexaethyl-violet) leads to the most active bactericidal substances known for staphylococcus, as originally shown by Dreyer, Kriegler and Walker[35] with methyl-violet, *B. anthracis* and *B. diphtheriae*. Morgenroth and his colleagues[36] have been especially interested in the chemical constitution of the acridin dyes in relation to bactericidal activity for streptococci and in tests employing serum-broth dilutions of these dyes found that the structure of the whole molecule may influence the function of the side arms, but that in the acridins the alkyl groups are the most active therapeutically and the isoamyl groups the least. As a result of these investigations "rivanol" (2-aethoxy-6, 9-diaminoacridin chlorhyrate) was evolved and found particularly effective in the treatment of mice inoculated subcutaneously with virulent streptococci.

Working with gentian-violet and magenta as "normal" triphenylmethanes and with acid fuchsin and the trisulfonic acid salt of rosanilin as representatives of "reverse selective activity," Churchman[37] has shown that the selective activity of these dyes is not to be ascribed to sodium. On the contrary however, the presence of SO_3 radicals appears to account for reverse selective activity. It would appear, therefore, as stated by Churchman, that with this group of dyes at least, the specific selective activity might "depend not so much on the presence of the sulfonic group as on the method of its bonding, though it is exhibited by substances like chromotropic acid, in which the SO_3 group is firmly attached, as well as by substances like sulfonic acid, in which the bonding is very weak. It is, however, well known that physical chemists are moving away from the ready explanation of phenomena of this sort on a purely chemical basis and are tending more and more to regard them as physical in nature."

Krigler,[38] whose work has already been referred to and the results of which also showed marked selective activities on the part of various organic compounds including some of the dyes, has also drawn several interesting conclusions on the relation of the chemical constitution of various organic substances including anilin dyes. Insofar as gentian-violet is concerned it is apparent that the crystal violet portion is more effective than the methyl violet and the degree of methylation would appear therefore, of importance in relation to the bactericidal effects which opens up the possibility of preparing a crystal violet with more than the six methyl groups of this dye.

Mechanism of the Bacteriostatic and Bactericidal Activity of the Anilin Dyes.—Just as we are lacking in sufficient information on the relation of chemical constitution of the anilin dyes to bacteriostatic and bactericidal activities to enable one to establish the highly desirable "laws" for aiding advances in our knowledge of the chemotherapy of these substances by synthesizing new compounds in a more orderly fashion, so are we likewise lacking in accurate information on the mechanism of their bactericidal activities. We ordinarily state that phenol, mercuric chlorid and other disinfectants kill bacteria by coagulating the proteins of their protoplasm but the process is probably not so simple and certainly is not in relation to the anilin dyes.

Genesistasis in Relation to Bacteriostasis.—As stated by Churchman[39] there is a good deal of reason for supposing that bacteriostasis may act by paralyzing the reproductive mechanism of bacteria without otherwise injurying the organisms; this is designated as "genesistasis" or the "genesistatic activity" of disinfectants. That powerful action on cell processes can be exerted by substances which do not penetrate beyond the surface of the cell has been shown by Warburg.[40] Churchman states there is evidence for the conclusion that gentian-violet has an inhibiting effect on the fission of paramecium even in dilutions which do not penetrate the cell or otherwise injure the organism; and in one instance he was able to observe a paramecium, lying in gentian-violet, begin its division but never conclude it—a striking example of genesistasis. Churchman believes that this phenomenon may be due to changes effected by the dye at the surface of the organisms and bear an important relation to the mechanism of genesistasis or bacteriostasis.

Penetration and Staining in Relation to Bacteriostasis.—As stated by Churchman, the simplest explanation for the behavior of gentian-violet and acid fuchsin toward bacteria is that the whole process is simply one of selective penetration. Penetration does not, however, "entirely account for the behavior of these dyes unless we interpret penetration to mean, not merely arrival of the dye within the bacterial membrane, but its arrival at some

particular portion of the bacterial body concerned with reproduction—a procedure which could not be made out with the microscope and which it is difficult to separate in thought from chemical affinity. It must, however, be remembered that when we speak of penetration by a dye we usually think of penetration by the colored portion of the dye since this is the only portion we can see. It might well be that the effective portion of the acid fuchsin molecule (let us say the SO_3 radical) penetrates Gram-negatives readily and kills them even though they are not stained, that is penetrated by the visible portion of the dye."

That static effects may be produced without penetration is, therefore, clear. But it is equally true that penetration of a dye may occur without bacteriostasis as shown in a splendid study by Simon and Wood[41] on the mechanism of the inhibitory action of certain anilin dyes upon bacterial development.

Ionization, Electrical Adsorption, etc., in Relation to Bacteriostasis.—According to Stearn and Stearn,[42] the staining reactions of all bacteria show that they behave as ampholytes tending to retain basic dyes in alkaline solution and acid dyes in acid solution. It might well be expected that the formation of a loose compound between any constituents of the media which are not easily decomposed by hydrolytic action, and thus "digested" by the bacteria, should interfere with the life cycles of such organisms. Bacteriostats, they believe, act in this way and that the behavior of bacteria toward dyes, so far as staining reaction is concerned, is well accounted for by their modification of Loeb's[43] theory of proteins applied to bacteria. On the acid side of the isoelectric point it might be expected that acid dyes could be just as effective as are basic dyes on the alkaline side. For a simple protein they might be, but they have pointed out that staining characteristics of bacteria indicate an isoelectric range between the isoelectric points of bacterial protein and bacterial lipoid, through which the protein would act as a base and the lipoid as an acid.

Basic dyes find their task very simple, for a solution on the alkaline side of the isoelectric point of bacterial protein is also on the same side of the isoelectric point of the much more strongly acidic lipoid, and there is thus no interference with action. Throughout the isoelectric range, however, the lipoid is working against an acid dye and even though the reaction is favorable for dye-protein combination it is also favorable for lipoid-protein combination into a conjugate protein, and we have both lipoid and acid competing for the protein and a very much greater quantity of acid dye would have to be used for the same inhibitive effect than of basic dye under the former set of conditions.

For acid dyes to have an effect commensurate with the well known effectiveness of basic dyes when used properly, it would be necessary to lower the pH to a value below the isoelectric point not only of the protein but also of the lipoid. In general this will throw the reaction over to an acidity so great that inhibition of growth will take place without the addition of dye at all, so that the practical application of high dilutions of acid dyes seems limited.

Churchman states that the method of ionization of the two groups of dyes whose action has been studied by him may explain their behavior. It is suggestive in his opinion that one group is basic and the other acid and that if this kind of explanation is the correct one it must be that Gram-positive spore bearers and Gram-negatives differ in their H ion concentration. He also mentions that since the basic dyes are electro-positive while the acid dyes are electro-negative that it is tempting to explain selective activity

on the basis of electrical absorption, an interesting thought deserving of further investigation.

Of additional interest in this connection is the work of Burke and Graves[44] on the influence of alkali in dye therapy indicating that bacteriostatic and bactericidal action of many of the dyes varies with the hydrogen ion concentration of the medium surrounding the organisms. With an increase in alkalinity there is, in their opinion, with but few exceptions, an increase in the action of dyes among which are crystal-violet, methyl-violet, gentian-violet, brilliant green, basic fuchsin, the flavins and malachite green. They believe that it is advisable, therefore, to attempt rendering the body fluids more alkaline by the administration of sodium bicarbonate or the application of an alkaline wash before a dye is applied in the treatment of localized infections.

Dye Fastness of Bacteria in Relation to Chemotherapy.—Of considerable interest in relation to the employment of dyes in the chemotherapy of bacterial infections is the question of whether or not some species may acquire or naturally possess a resistance or fastness to the anilin dyes. Simon and Wood believe that some members belonging to dye-susceptible groups of organisms exist in nature which are not inhibited by the dyes in question and that dye susceptibility may be overcome to a greater or less extent by adaptation or the acquisition of fastness (resistance).

Shiga[45] succeeded in rendering cholera bacilli resistant or fast to some dyes and Churchman has worked with strains possessing an impeccable fastness to gentian-violet, but Browning and Gulbranson[46] have stated that they were unable to produce fastness or resistance with acriflavin and brilliant green and Gay and Morrison[47] state that although their experiments are not extensive on this point, they are unequivocal and show clearly that repeated subcultures of the streptococcus in the highest non-bactericidal dilution of several of the most active dyes not only did not render the microörganisms more resistant to more concentrated solutions of the dye, but actually seemed to develop a greater susceptibility to the dye in dilutions in which it originally grew.

Various spirochetes and trypanosomes, however, have been rendered resistant to anilin dyes, arsenicals and other substances and my own experience indicates that different strains of staphylococci, streptococci, colon and typhoid bacilli may exhibit slightly varying natural resistance to the anilin dyes; I have not tried to render any one strain particularly resistant or fast to a particular dye, but am prepared to believe that this is possible by prolonged and careful experiments in which the organisms are exposed to progressively increasing but subbactericidal strengths of the dye.

Influence of the Anilin Dyes upon Phagocytosis and Antibodies.—Numerous questions naturally arise when the dyes are employed for the treatment of local or general bacterial infections in relation to their influence upon phagocytosis, antibodies and immunological reactions. For example, may these substances applied locally to infected wounds or serous membranes inhibit phagocytosis of the organisms by leukocytes? May they destroy complement and bacteriolysins by local application or intravenous injection? May they increase antibody production upon intravenous injection or absorption from the subcutaneous tissues, muscles or gastro-intestinal tract? These questions are not without interest and some practical importance in relation to the use of the dyes for therapeutic purposes.

Naturally most interest has been commanded by the influence of dyes on phagocytosis since they have been generally employed in the treatment of local infections of wounds and serous cavities. Browning and Gulbranson[48]

found that concentrations of 1:7000 malachite green and crystal-violet, 1:2000 brilliant green and 1:500 acriflavin, inhibit phagocytosis, the last mentioned being the least anti-phagocytic of the group tested and from this standpoint most desirable for the treatment of wounds by topical application. Fleming[49] and Morgan[50] have also stated that acriflavin destroys leukocytes but Davis[51] affirms that phagocytosis is still active in concentrations of the dye which are two hundred times as concentrated as the bactericidal dilution. Gay and Morrison report that final concentrations of acriflavin 1:2400 or stronger appeared to slightly depress the phagocytic activity of leukocytes for streptococci in the test tube while weaker solutions probably increased phagocytosis slightly or at least did not depress the activity of the cells. Meleney and Zau[52] however, found that intravenous injections of large doses of acriflavin to rabbits caused a leukocytosis in direct proportion to the dose; that the dye had little or no effect on the number or fragility of erythrocytes and coagulation or bleeding time and that it exerted a temporary deleterious effect on the functional activity of the polymorphonuclear leukocytes.

With the assistance of Miss Richter, I have studied the influence of solutions of various dyes upon the complement of the guinea-pig and we found the results applicable for estimating the probable effect of the dyes upon the complement to be found in exudates and in the plasma of the blood. Final concentrations of 1:100 to 1:1600 of acriflavin, gentian-violet, methylene blue and the fuchsins were in general destructive, but since the dyes are usually employed for local application in 1:1000 solutions and since further dilution occurs after contact with secretions, it would appear that appreciable or harmful degrees of complement destruction do not ordinarily occur and the intravenous injection of amounts varying from 0.005 to 0.010 gm. per kilogram of weight had no effect at all upon the complement of the plasma. Additional experiments have shown that the amboceptors, to which the bacteriolysins belong, are likewise susceptible. For example, the dyes mentioned in final concentrations varying from 1:200 to 1:600 sometimes proved destructive for hemolysin and while the results of additional experiments employing a bacteriolysin (cholera immune serum) were not as sharp or definite, yet they were essentially similar. These experiments indicate that it is not advisable to use the dyes in too high concentration for the local treatment of infected wounds, serous cavities, etc. The natural tendency is to employ them in the strongest solutions free of manifest irritation for the tissues but this practice may tend to defeat their curative activities by preventing the antibacterial properties of phagocytosis and bacteriolysis, the influence of which should not be under estimated.

May the intravenous injection of the dyes increase antibody production? A large amount of work has been done with trypan-red and other dyes in relation to antibody-production in trypanosomiasis, but the subject has not commanded interest in relation to bacterial infections although at the present time both acriflavin and gentian-violet are being injected intravenously in the treatment of bacteremias by streptococci, staphylococci and other organisms.

In my experiments I have not found acriflavin, gentian-violet or acid fuchsin, in dose of 0.010 gm. per kilogram by intravenous injection every five days for 4 to 6 doses, to appreciably influence either the complement of the blood of rabbits, natural antibody (anti-sheep hemolysin) or immune antibodies (typhoid bacteriolysin and agglutinin). When due allowance was made for natural variations among animals and unavoidable experimental error, the control animals showed approximately the same amounts

of complement and antibody in the blood as the animals treated with the dyes. It was observed that gentian-violet, acriflavin and acid fuchsin at least were without effect in stimulating the hemolysin producing tissues of the rabbit to greater activity and similar results were observed in a set of animals immunized by intravenous injections of cholera vaccine. It may be that a part of the bactericidal effect of gentian-violet and acriflavin injected intravenously in the treatment of bacteremias by streptococci and staphylococci, is due to increased leukocytosis as well as the mobilization of various enzymes, but I doubt that the dyes actually increase antibody or complement production by stimulation of the bone marrow and other tissues believed to be concerned in their production.

Toxicity and Pharmacological Action of the Anilin Dyes in Relation to Chemotherapy.—Since some of the anilin dyes and especially gentian-violet, rivanol, acriflavin and proflavin are sometimes injected intravenously in the treatment of streptococcus, staphylococcus and other bacteremias of human beings, it is important to enquire into the matter of their toxicity and pharmacological action. As would be expected in view of the complicated chemistry and preparation of these substances, toxicity varies considerably with the different dyes and indeed, with different lots of the same dye prepared in different laboratories. It is imperative in the interests of safety to select for intravenous injection only those dyes especially prepared for intravenous injection by processes of purification to remove the numerous impurities which may be present and dyes suitable for ordinary bacteriological work may be quite too toxic and unsuitable for intravenous injection.

Toxicity of Gentian-violet, Acriflavin, Rivanol and Other Anilin Dyes for the Lower Animals by Intravenous Injection.—Churchman and Herz[53] state that rabbits sometimes stand doses of *gentian-violet* as large as 10 c.c. of 1:200 solution by intravenous injection without apparent immediate or subsequent ill effects; this dose may be as large as 0.025 gm. per kilogram of weight, corresponding to over 1.5 gms. for an adult human being of about 140 pounds. In other experiments, however, these injections have produced violent convulsions and death with pulmonary edema or no discernible changes other than staining of the tissues. The authors do not state whether these effects were produced by the same lot of dye or by gentian-violet prepared by two different laboratories. If produced by the same dye it is indeed difficult to explain the phenomenon unless some animals possess an unusual natural susceptibility to the dye. However, if produced by gentian-violets of different manufacture it is not difficult to explain the results, since I have found one gentian-violet withstood by rats without apparent ill effects in dose higher than 0.1 gm. per kilogram, whereas the dye prepared by a second laboratory produced acute convulsions and death in dose of about 0.025 gm. per kilo, approximately the amount injected by Churchman and Herz.

Young and Hill[54] report that the intravenous injection of normal rabbits with gentian-violet killed in dose of 0.020 gm. per kilogram in five hours. However, single doses of 0.005 to 0.010 gm. per kilogram have been well tolerated in every instance. Repeated injections, given three times a week, have shown that of three rabbits given 0.005 gm. per kilogram, two died after the seventh injection, one tolerating seven injections well. Two rabbits died after four doses of 0.0075 gm. per kilogram. Of four rabbits given 0.010 gm. per kilogram, one died after the fifth dose, one after the fourth, and two after the second, an average of about three doses.

Spencer[55] reports that the maximal tolerated dose of neutral *acriflavin* for the mouse was about 0.4 c.c. of 1:5000 per 20 grams of weight; this corresponds to 0.004 gm. per kilogram which is much more toxic than I have

found neutral acriflavin to be for rats by intravenous injection. Meleney and Zau state that with lethal doses of neutral acriflavin administered to rabbits by intravenous injection, death is due to profound degenerative changes in the convoluted tubules of the kidney and to central degeneration in the liver.

Morgenroth and his associates state that the maximum tolerated dose of rivanol for mice is 0.3 c.c. of a 1:200 solution per 20 gm. of body weight by subcutaneous injection and corresponding to 0.075 gm. per kilo. By intraperitoneal injection the dose is 0.5 c.c. of 1:600 solution and by intravenous injection 0.5 c.c. of a solution 1:1500, corresponding respectively to 0.04 and 0.016 gm. per kilogram. The immediate lethal dose for a rabbit is 0.050 gm. per kilo intravenously and 0.1 gm. subcutaneously. Resorption from the tissues takes place slowly. Death occurs from cessation of breathing. The rapid intravenous injection of 0.010 gm. produces temporarily a marked depression of the blood pressure in a rabbit. The effect of the same dose administered slowly is less intense. In the subcutaneous tissues of mice and horses solutions of 1:500 to 1:1000 are tolerated without any injurious effect on the tissues. More concentrated solutions cause infiltrations.

My own toxicity tests, conducted with the assistance of Miss Anna Rule, and employing white rats of 100 to 200 grams weight, have yielded the following results expressed in terms of the *maximum tolerated doses* over a period of at least two weeks. The solutions varied from 0.5 to 1 per cent. and were injected at a constant rate of about 2 c.c. per minute as described in Chapter II.

Gentian-violet (C and B)	0.125 gm. per kilogram
Gentian-violet (N)	0.025 gm. per kilogram
Acriflavin (N)	0.035 gm. per kilogram
Acid fuchsin (C and B)	0.130 gm. per kilogram
Basic fuchsin (C and B)	0.025 gm. per kilogram
Methylene-blue (C and B)	0.050 gm. per kilogram
Rivanol	0.050 gm. per kilogram

It is interesting to note that acid fuchsin prepared in saline solution is generally less toxic than basic fuchsin prepared in distilled water. All of the dyes produce staining of the tissues and it is stated that some, especially fluorescein, are more toxic when animals are exposed to sunlight. Proflavin is stated to be about half as toxic as acriflavin.

Toxicity of the Dyes for the Lower Animals by Intrapleural and Intracisternal Injection.—Since gentian-violet and acriflavin have been injected into the pleural cavities in the treatment of pleuritis, I have determined the toxicity of these in addition to acid and basic fuchsin and methylene-blue by this route of administration. Normal rats and rabbits were employed and the general results have been that all of these dyes were approximately 0.5 per cent. more toxic by this route than by intravenous injection.

I have also employed some of the dyes in the chemotherapy of experimentally produced streptococcus and staphylococcus meningitis of rabbits; toxicity tests employing normal rabbits injected intracisternally showed that the maximum tolerated dose of gentian-violet was from 0.001 to 0.002 gm. per kilogram, being at least ten to twenty times more toxic by this route than by intravenous injection. The maximum tolerated dose of acriflavin was between 0.002 to 0.003 gm. per kilogram and methylene-blue and acid fuchsin were tolerated in about the same or slightly larger doses. The total amount of fluid injected was about 0.25 c.c. per kilogram, as this amount of saline solution does not produce untoward effects by pressure and is a

convenient amount to inject by this route. Subdural injections in the lumbar region of rabbits and through trephine openings of the skull over the frontal lobes of the cerebrum yielded approximately the same results.

Mechanism of the Toxicity, Fate, Elimination and Pharmacological Action of the Anilin Dyes in the Lower Animals.—Comparatively little is known of the pharmacological action of the anilin dyes, largely because their use in the treatment of disease has been very limited until the last few years. Methylene-blue administered by subcutaneous or intravenous injection may cause a slight increase of blood-pressure by cardiac stimulation and is rapidly reduced to colorless leuko-compounds. It is excreted in the urine, feces, sweat and sputum, partly unchanged and partly as "methylene-azure," and has been injected subcutaneously in dose of 0.02 to 0.05 gm. as a test for renal function, the dye being normally excreted in fifteen to twenty-five minutes. All of the dyes appear to be excreted largely in the bile and urine, but the rapidity of elimination varies considerably. Beckwith[56] who has studied *in vitro* the effects of bile, serum, erythrocytes and emulsions of the brain, muscle, lung, marrow, liver, spleen, and kidney upon the absorption and decolorization of 22 different dyes in relation to their fate *in vivo* noted that little change was wrought in any of the dyes tested. On the other hand, he believes that certain secreting and filtering tissues, such as liver, kidney, bone marrow and spleen, show a high degree of activity, lung and muscle occupying an intermediate position. Certain dyes such as acriflavin and proflavin showed no change throughout. Other stains varied in outcome according to the particular cell structure with which they were placed in contact. Some dyes were reduced to the leuko-base as methylene-blue many times and as trypan-blue once. Others were changed into products further removed from the original molecular structure. It was noted that while one dye may be eliminated in part through the bile, another may be found only in the urine. This of itself denotes a selective affinity in the first coloring matter for special cell structures which does not exist in the second dye.

Acriflavin in toxic doses by intravenous injection is said to kill by paralysis of respiration and I believe that a combination of cardiac and respiratory paralysis accounts for the acute deaths of rabbits and other of the lower animals injected intravenously, intracisternally or intrapleurally with toxic amounts of the anilin dyes.

Of considerable interest in relation to the mechanism of toxic reactions following intravenous injections of the dyes, is the question of their hemolyzing and agglutinating effects upon the erythrocytes. As discussed in Chapter XLV, these effects are regarded as important in relation to the toxic reactions following the administration of arsphenamin and other organic arsenicals, and the subject always commands attention when drugs are injected intravenously, as well as the possibility of their producing precipitates with the proteins of the plasma. With the assistance of Miss Richter, I have studied these effects with interesting results as follows:

Dye	Hemolysis (Washed Cells)	Hemolysis (Unwashed Cells)
Acriflavin	1:200 (slight)	not in 1:100
Gentian-violet	1:12,000	1:800
Acid fuchsin	1:400 (slight)	not in 1:100
Basic fuchsin	1:200 (slight)	not in 1:100
Methylene blue	1:100 (slight)	not in 1:100

The results of the *hemagglutination* and *serum precipitation* tests were as follows:

Dye	Agglutination (Washed Cells)	Agglutination (Unwashed Cells)	Precipitation
Acriflavin	1:3200	1:400	not in 1:500
Gentian-violet	not in 1:100	not in 1:100	not in 1:500
Acid fuchsin	1:800	not in 1:100	not in 1:500
Basic fuchsin	1:800	not in 1:100	not in 1:500
Methylene blue	1:800	1:100	not in 1:500

Bohland[57] states that acriflavin agglutinates erythrocytes and Spencer[58] found it hemolytic in dilutions as high as 1:4000 to 1:8000 but free of agglutinating activity for unwashed human corpuscles in dilutions of 1:2000 and higher. Spencer also observed that slight hemolysis occurred *in vivo* within an hour after the intravenous injection of rabbits with 0.1 gm. per kilogram of weight.

From the standpoint of a possible relationship between intravascular hemolysis and hemagglutination and acute toxic reactions, most interest is commanded by the results observed with unwashed corpuscles since it is apparent that serum and doubtless the plasma, afford a great deal of protection against both reactions. Acriflavin has proved most agglutinative and gentian-violet most hemolytic, but both effects are relatively slight in the presence of serum and I doubt if these reactions are produced *in vivo* by the amounts ordinarily given or exert any important rôle in the production of toxic reactions similar, for example, to hemolysis and hemagglutination in relation to the acute toxic reaction following the intravenous injection of acid solutions of arsphenamin. At least, additional experiments conducted by injecting the dyes intravenously into rabbits in dose of 0.020 gm. per kilogram and examining the blood of the right auricle and lungs did not reveal any evidences of hemagglutination or hemolysis *in vivo* and all tests for precipitation of proteins yielded negative results.

The acute toxic reactions of the anilin dyes appear, therefore, to be largely due to an effect upon the higher nerve centers and especially the cardiac and respiratory centers; the chronic effects due to repeated injections are probably due to injuries of the liver and kidneys as suggested by the results of a preliminary study I have recently made of the histo-pathological changes in rabbits treated with six to twelve intravenous injections of gentian-violet, acriflavin and basic fuchsin in dose of 0.005 gm. per kilogram every five days.

Toxicity of the Dyes for Human Beings.—With this review of the toxicity of some of the anilin dyes for the lower animals, their toxicity for human beings may now be discussed. Most interest is commanded by gentian-violet, acriflavin, proflavin, methylene-blue, trypan-red and rivanol, since these have been generally employed in attempts to successfully combat bacterial infections.

The dose of *gentian-violet* commonly administered by intravenous injection is 0.005 gm. per kilogram corresponding to 0.3 to 0.4 gm. for an adult of average weight (30 to 40 c.c. of a 1 per-cent. solution). As a general rule, 20 to 30 c.c. of a 1-per cent. solution has been given and this dose is fairly safe, being at least 5 to 10 times less than the maximum tolerated dose for the lower animals. Following the injection the skin and mucous mem-

branes become a dusky blue in color, suggesting severe cyanosis, but due to the dye, and usually disappearing within a few hours. The breathing and pulse rate may be increased with a slight increase of blood-pressure; occasionally the patient experiences a tingling sensation of the skin with abdominal uneasiness, but I have never observed any other untoward effects. It may be that some individuals are more susceptible than others but I have not encountered any so far among more than sixteen adults to whom I have given one to three injections of Coleman and Bell's gentian-violet in dose of approximately 0.005 gm. per kilogram in 1 per cent. solution *slowly* injected.

Neutral *acriflavin* and *proflavin* are generally administered in dose of 0.005 to 0.010 gm. per kilogram corresponding to 0.3 to 0.7 gm. for an adult of average weight; usually the dose is 30 to 50 c.c. of a 1-per cent. solution (0.3 to 0.5 gm.) by *slow* intravenous injection. The skin may become flushed and especially the face; both cardiac and respiratory rates may be increased for a few minutes but in the majority of cases there are no other effects. These doses are at least 4 to 5 times less than the maximum tolerated doses for the lower animals and thereby within the range of safety but it is my practice never to inject more than 20 c.c. of a 1-per cent. solution (0.2 gm.) for the first dose when the patient is profoundly toxic, since resistance is materially reduced by severe bacterial infections.

Methylene-blue has been generally administered by injestion in dose of 0.15 gm. (2½ grains) without untoward effects.

Trypan-red has been injected intravenously in dose of 1 gm. in a 0.2 per cent. solution without ill effects; I have had no experience with it in the treatment of human subjects.

Rivanol is usually employed by local application to wounds but according to Morgenroth, may be administered intravenously with safety in dose of 30 to 50 c.c. of 1:1000 solution for adults, corresponding approximately to 0.0005 to 0.0008 gm. per kilogram or about 20 to 30 times less than the maximal tolerated dose.

By local application in solutions of 1:500 to 1:1000 strength the dyes are well borne by wounds and such serous membranes as the pleural, subarachanoid and joint cavities. I have repeatedly injected solutions of these strengths into these parts of normal rabbits without finding signs of irritation in the way of exudates or such histological evidences as hyperemia, serous and cellular exudations. Stronger solutions however, while borne insofar as duration of life is concerned, may produce varying degrees of hyperemia and cellular exudations and especially of the meninges following subdural injections.

Mechanism of Curative Activity of the Anilin Dyes in Bacterial Diseases. Before taking up the question of the actual curative properties of the anilin dyes in bacterial infections of man and the lower animals it may be well to briefly summarize our present knowledge of the mechanism of their antibacterial properties when applied locally to infected membranes or injected intravenously.

Doubtless their curative effects are mainly due to direct penetration of organisms followed by such changes in the life processes as results in an interference with proliferation (bacteriostasis) or death (bactericidal). Just how these effects are produced, however, is not known; possibly there is an interference with vital activities by processes of oxidation and reduction or by the production of new compounds interfering with metabolism. In the treatment of pleuritis and other infections of closed serous cavities it is usually possible to introduce sufficient dye to raise the concentration in the

pus to bactericidal or bacteriostatic proportions but these curative effects may be neutralized by rapid fixation of the dye by tissue cells and before appreciable numbers of organisms are reached and destroyed. For this reason the parts must first be cleansed of pus before application of the dye in order to spare as much as possible for the destruction of bacteria. Likewise the intravenous injection of 0.010 gm. per kilogram may hardly raise the concentration in the blood to bactericidal proportions and especially since the dyes tend to be reduced or otherwise rapidly removed from the blood stream. But acting as adjuvants to serum therapy or administered by daily injection, some curative effects may result.

Furthermore, when the dyes are injected intravenously certain non-specific effects may aid in the curative activity. For example, the injection of rabbits with gentian-violet in dose of 0.010 gm. per kilogram usually results in a leukocytosis of moderate degree within an hour, mainly due to a total increase of the polymorphonuclear neutrophilic leukocytes, lasting for at least two hours and reaching normal proportions in twenty-four hours. Spencer reports that the intravenous injection of rabbits with acriflavin in dose of 0.1 gm. per kilogram results within three hours in a leukocytosis of over 100 per cent. which was only somewhat reduced by the end of twenty-four hours.

The serum proteases, however, are not constantly or markedly increased. My student, Dr. Ling, found the following values in normal rabbits injected intravenously with gentian-violet in dose of 0.010 gm. per kilogram, the results being expressed in terms of the number of milligrams of non-coagulable nitrogen per 100 c.c. of serum after 48 hours digestion:

	Rabbit No. 1	Rabbit No. 2	Average
Before injection	1.4	0.7	1.05
4 hours after injection	1.4	1.4	1.4
24 hours after injection	0.7	0.7	1.05

The slight increase noted four hours after injection may represent an actual result of the dye but the results are practically within the range of normal variations for rabbits.

Anilin Dyes in the Chemotherapy of Experimentally Produced Local and General Bacterial Infections.—But comparatively little work has been done with the anilin dyes in the treatment of experimental bacterial infections of the lower animals except in tuberculosis, although they have been quite widely employed in the treatment of local and systemic infections of human beings. It is true that experimentally produced infections are usually severe and place a heavy strain upon the curative activities of a medicament, but yet the logical procedure is to first apply studies of this kind, whenever possible and before clinical application is made in order to avoid the pitfalls of hasty and erroneous conclusions regarding the practical curative activity of any agent under study.

Anilin Dyes in the Chemotherapy of Experimental Staphylococcus, Streptococcus and Pneumococcus Infections.—A notable example of orderly investigation is afforded by the work of Morgenroth and his associates[59] with acridin derivatives and especially rivanol, in experimental staphyloccoccus and streptococcus infections. As described in Chapter IV, they have worked out a method for producing deep streptococcus and staphylococcus abscesses in mice by the subcutaneous injection of small amounts of culture and have used these experimentally produced infections for studying the curative

effects of new drugs *in vivo*. They report that tests with as many as forty different strains of streptococci have shown that the injection of mice with 1 c.c. of 1:40,000 solution of the drug (approximately 0.00125 gm. per kilogram) is sufficient for destroying these organisms within twenty-four hours; in staphylococcus infections the required dose is about twice as large or 1 c.c. of 1:20,000 solution (0.0025 gm. per kilogram). Hussey[60] states that silver and gold salts with the acridins have proved curative in experimental streptococcus and anthrax infections and that the silver compounds are of clinical value in the treatment of streptococcus bacteremia, gonococcus infections and polyarthritis.

Gay and Morrison[61] have reported upon some of the dyes and especially acriflavin, in the treatment of experimentally produced streptococcus pleuritis and empyema of rabbits but do not believe that the outlook for developing a dye therapy for infections of closed cavities is very promising; it is to be remembered, however, in this connection, that no attempt was made in their experiments to first drain and cleanse the pleural cavities before the introduction of the dye, as advised and emphasized by Churchman[62] in his work upon the dye therapy of infected joints and pleurae with gentian-violet. Meleney and Zau[63] state that neutral acriflavin injected intravenously failed to favorably influence streptococcus infections of rabbits and Hirschfelder and Winternitz[64] failed to influence the course of experimental pneumococcus pneumonias of rabbits treated with trypan-blue, trypan-red, basic fuchsin, acid fuchsin, crystal-violet, malachite green, gentian-violet, erythrosin and other substances like salvarsan, sodium antimony thioglycollate, sodium iodo-o-oxy-quinolin sulphonate, potassium guaracolsulphonate (thiocol), hexamethylenamin, etc.

With the assistance of Dr. Malcolm J. Harkins, I have investigated the curative properties of acriflavin and gentian-violet in mice, rats and rabbits infected with virulent streptococci, staphylococci and pneumococci. Mice were inoculated subcutaneously according to Morgenroth's method and the dyes injected subcutaneously or intraperitoneally, the first dose being given four hours after infection and subsequent doses at daily intervals. Controls were of course included on the cultures alone and the two dyes. Gentian-violet was given in dose of 0.005 to 0.010 gm. per kilogram and neutral acriflavin in dose of 0.010 gm. per kilogram. The untreated controls generally succumbed in forty-eight to seventy-two hours with well-developed local lesions in the streptococcus and staphylococcus infections. The streptococci and anthrax bacilli were also usually to be found in the blood of the heart. The results observed among the treated animals were largely negative; in some instances, not more than 10 per cent. of the animals, some appreciable effects were observed in the prolongation of life for one or two days beyond the survival of the controls and the effects of both dyes were approximately similar.

Since white rats possess a higher natural immunity to streptococci, they were infected by the intravenous injection of 0.5 c.c. of a twenty-four-hour broth culture of a highly virulent hemolytic strain of streptococcus. The two dyes were injected intravenously or intraperitoneally in dose of 0.005 to 0.010 gm. per kilogram, the first dose being given four hours after infection. The results, however, were negative since the treated animals succumbed with a streptococcus bacteremia in about the same time as the untreated controls (twenty-four to forty-two hours).

Rabbits were infected by daily intravenous injections of 0.5 c.c. of 1:1,000,000 dilution of twenty-four-hour broth cultures of this highly virulent streptococcus in an attempt to produce a milder and more prolonged bactere-

mia. Both dyes were injected intravenously in dose of 0.005 to 0.010 gm. per kilogram, each dose being given four hours after inoculation. The controls generally survived for four to five days, succumbing with bacteremia and yielding positive blood cultures during life and heart cultures after death. Neither dye appreciably influenced the duration of life and when due allowance was made for individual variations probably due to natural resistance, our results were interpreted as negative.

With the assistance of Miss Anna Rule, I have also produced acute suppurative meningitis in rats, guinea-pigs, dogs and rabbits by the subdural or intracisternal injection of virulent streptococci, pneumococci and staphylococci. Gentian-violet and neutral acriflavin were administered by intracisternal injection in dose of 0.0001 to 0.002 gm. per kilogram, the first dose being given four hours after infection and subsequent doses at daily intervals. As a general rule, however, the results were negative in that the treated animals succumbed in about the same time as the untreated controls, showing cloudy spinal fluids containing enormous numbers of cocci and in the streptococcus infections, with the organisms in the heart blood. Animals receiving as many as six to ten injections of the drugs alone, however, survived without appreciable harmful effects, indicating that both dyes in the doses given were well tolerated by this route of administration.

We have also worked with rivanol but were unable to demonstrate any constant or well defined curative activities in these experimental infections and I believe the same applies to other anilin dyes of the triphenylmethane series. Mention may also be made that Spencer found acriflavin unable to check or control pneumococcus infections of mice. Churchman[65] has never been able to kill organisms circulating in the blood by intravenous injections of gentian violet and while there is some evidence in the literature that it may at some time prove possible to produce a *sterilisans magna* in this way, certainly at the present time there is nothing to suggest that it is now possible to do for bacteria what quinin does for malaria.

Anilin Dyes in the Chemotherapy of Experimental Infections Produced by Typhoid Bacilli and Other Gram-negative Bacilli.—Since acid and basic fuchsin have been shown by Churchman to possess a reverse selective activity for Gram-negative organisms and especially *B. coli*, it would appear that these dyes as well as acriflavin and others found to possess a high bactericidal activity for typhoid bacilli *in vitro*, may be worthy of study in relation to the chemotherapy of infections produced by typhoid and paratyphoid bacilli, colon, cholera, dysentery and other bacilli of this large Gram-negative group. Churchman has found that the local application of solutions of fuchsin to wounds infected with the Gram-negative *B. pyocyaneus* may remove these organisms, but I have not been able to locate any reports bearing upon the dye therapy of localized lesions experimentally produced by Gram-negative organisms, either bacilli or cocci except the investigation by Beckwith,[66] on the chemotherapy of experimentally produced typhoid carriers in rabbits. He succeeded in producing this state by the method developed by Gay and Claypole[67] and investigated the curative activity of a number of dyes but found them ineffective for sterilizing gall-bladders, containing *B. typhosus* in the living body. New fast green 3B offered possibilities because it retains its activity in serum and in bile and is excreted through the bile when administered intravenously, but this dye was not effective in clearing up the carrier condition of all animals. Uhlenhuth and Messerschmidt,[68] working with rabbits rendered typhoid carriers by direct gall-bladder inoculation, state that while spontaneous cures occur in about three per cent. yet the intravenous and intramuscular injection of methyl violet stains the bile

and upper intestinal tract and cures the majority of animals; negative results occurred with the gold, mercury and copper salts of fluorescein and safranin.

Dr. Harkins and I have been experimenting with typhoid and colon infections of rabbits treated with intravenous injections of acid and basic fuchsin, acriflavin and other dyes, but owing to the difficulty of producing sufficiently uniform infections and especially bacteremias, our therapeutic results are too difficult of proper evaluation to warrant any statement at the present writing. Our work is being continued however, because it may be quite possible to develop a dye therapy for local colon bacillus infections as well as for typhoid and paratyphoid fever; indeed, as will be discussed in the succeeding chapter, mercurochrome injected intravenously, has yielded encouraging results in the treatment of *B. coli* abscesses and the dyes may also prove of value in these and similar infections.

Anilin Dyes in the Chemotherapy of Experimental Infections Produced by Tubercle, Anthrax and Other Gram-positive Bacilli.—Naturally a large amount of investigation has been devoted to the subject of the chemotherapy of experimental tuberculosis, infected guinea-pigs and rabbits (cornea) being usually employed; an excellent account of these studies is given by Wells, DeWitt and Long.[69] DeWitt has kept guinea-pigs infected with tubercle bacilli, saturated for about two months with trypan-red and trypan-blue; they lived somewhat longer than the controls but ultimately died of generalized tuberculosis. Since these dyes penetrate the tubercle so well, it was thought they might be used to carry in some more effective bactericidal agents and trypan-blue was therefore combined chemically with silver, copper, iron and mercury. Only the two mercury salts showed increased bactericidal action, the guinea-pigs inoculated with tubercle bacilli which had been exposed to these developing neither local nor general tuberculosis. Treatment of tuberculous guinea-pigs with these metal compounds of trypan-blue, copper, iron and silver salts, failed to affect the progress of the disease, while the animals treated with the mercury salt lived somewhat longer and had much less tuberculous involvement. Hence DeWitt concluded that trypan-blue and trypan-red, either as the sodium salts or as any one of the metal salts used in her experiments have failed to kill the tubercle bacilli either in the test tube or in the guinea-pig. Lewis and Kraus have also produced a large number of new compounds of trypan-red or trypan-blue combined with iodin, phenol and other substances and while some appeared to influence the vigor and rate of formation of blood-vessels and connective tissue around tubercles in the eyes of rabbits and while some were developed capable of penetrating tubercles, sometimes more or less specifically, none have cured tuberculosis in guinea-pigs. Von Linden[70] has described rather encouraging therapeutic results in six guinea-pigs inoculated with tubercle bacilli and treated with daily subcutaneous injections of methylene-blue iodid in dose of 0.2 to 0.4 c.c. of a 1:1000 solution, but DeWitt[71] has made an especially extensive investigation of methylene-blue chlorid and iodid and new compounds of these dyes and observed that they sometimes modified the disease and prolonged the lives of the animals but yet in no single case was tuberculosis cured by any of them.

None of the dyes so far tested, therefore, have shown any definite therapeutic power in experimental tuberculosis in guinea-pigs beyond some prolongation of life, and some lessening of the severity of the disease, although the penetrating and bactericidal or bacteriostatic powers were good. It is still possible, however, that some of the easily diffusible dyes may be used as carriers for more efficient therapeutic substances, which may reach in this way the tuberculous tissue which they could not otherwise attain.

Petroff[72] found acriflavin and proflavin by subcutaneous injection without effect in the treatment of guinea-pigs inoculated with a highly virulent culture and Smith[73] observed that while acriflavin, proflavin, and their silver compounds, acridinum-yellow, and acridin-orange possess a high degree of inhibition on the growth of the tubercle bacillus *in vitro*, they do not alter the pathogenicity of the tubercle bacillus when exposed to their action at 38°C. for forty-eight hours. Furthermore, experiments made to determine whether the serum of animals treated with proflavin would show inhibiting or bactericidal properties for the tubercle bacillus yielded negative results and proflavin, acriflavin, and the silver compound of the latter applied in the treatment of experimental tuberculosis in guinea-pigs and rabbits had no beneficial effects on the tuberculous processes.

Comparatively little work has been reported upon in the chemotherapy of anthrax, tetanus, gas bacillus and other infections by Gram-positive bacilli. Since gentian-violet and other triphenylmethane dyes show such well marked selective bacteriostasis for Gram-positive spore bearing bacilli, it may be expected that these dyes may favorably influence infections produced by these organisms. Dr. Harkins and I have observed that gentian-violet administered intravenously and intraperitoneally to mice and rabbits infected with virulent anthrax exerts some degree of curative activity by lessening temporarily the severity of the bacteremia with prolongation of life, but so far we have not been able to cure animals in which the infection was given a twenty-four hour or longer start. The results are sufficiently encouraging, however, to warrant our advising the intravenous injection of gentian-violet in the treatment of those cases of human anthrax showing the bacilli in the blood, which always give a very grave prognosis; it may be also worth while to apply a dressing of gentian-violet to malignant pustules and especially after excision. Neufeld and his colleagues[74] state that brilliant green, crystal and methyl violet and dahlia have yielded encouraging results in experimental anthrax infections; acridin orange and aurophosphin were likewise slightly effective in experimental chicken cholera infections.

The dyes have been used in the treatment of experimental *B. tetani* infections, but with negative results; I have not been able to find, however, any records of work devoted to the chemotherapy of experimental infections by *B. welchii, B. maligni edematis* or other Gram-positive spore bearers producing wound infections. This would appear to be, however, a field worthy of investigation.

Anilin Dyes in the Chemotherapy of Bacterial Diseases of Man.—I shall reserve for Chapter VIII a more complete discussion of the practical value of the anilin dyes in the treatment of various bacterial infections of human beings, but a general statement of the applications and reported results may be proper at the present time.

Naturally the dyes have been mostly employed for the disinfection of wounds and localized infections of mucous membranes. Thus malachite green, brilliant green, gentian-violet, fuchsin, acriflavin, proflavin, rivanol, etc., have been employed for the treatment of infected wounds and apparently with some success although thorough preliminary cleansing and especially with alkaline solutions are required for the best results. It may be stated, however, that the dyes have not yielded as good results as Dakin's solution in infections of this kind.

Gentian-violet, brilliant green, acriflavin, etc., have also been employed for the disinfection of the throat and nose of diphtheria bacilli and meningococci, but with indifferent success, largely due to the difficulty of removing

the mucoid secretions sufficiently well to permit the penetration of the dyes into crypts where the organisms are especially likely to persist.

Acriflavin, proflavin and other dyes have also been employed in the treatment of infections of such mucous membranes as those of the mouth (in gingivitis), the eye, the urethra and vagina. Success is claimed by some and denied by others but many of the reports are of a favorable character.

Gentian-violet and acriflavin have also been employed for the treatment of pleuritis and infections of the larger joints and the results have been mainly good when the pus was first removed and the infected tissues cleansed before the injection of the dyes. This would appear to be quite a promising field for dye therapy and I believe there are also encouraging possibilities in the treatment of those dreaded and almost invariably fatal forms of meningitis caused by streptococci and pneumococci as well as of peritonitis caused by these and other organisms. Morgenroth and others have also reported the successful treatment of furuncles, carbuncles, buboes, suppurative mastitis, pelvic abscesses, etc., with acridin dyes and especially rivanol.

Gentian-violet, acriflavin, rivanol and other dyes have been used with apparent success in the treatment of staphylococcus and streptococcus bacteremias; indeed these would appear as special diseases calling for chemotherapeutic measures alone or as adjuvants to serum therapy and some notably successful results have been recorded.

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CHAPTER VI

ETHYLHYDROCUPREIN (OPTOCHIN) AND VARIOUS CINCHONA DERIVATIVES IN THE CHEMOTHERAPY OF PNEUMOCOCCUS AND OTHER BACTERIAL DISEASES

While the anilin dyes were among the first substances to be used for selective bacteriostasis and bactericidal activity and especially *in vitro*, in relation to the isolation of organisms, yet to cinchona derivatives belongs the distinction of being first employed for systematic efforts in the development of an efficient chemical disinfection of animals with an experimentally produced generalized bacterial infection. It has been long and generally known that quinin possesses certain bacteriostatic and bactericidal properties, and there is a long-standing tradition, endorsed by such clinicians of an older day as DaCosta and Bartholow,[1] that quinin given sufficiently early and in sufficiently large dose, will arrest an attack of pneumonia. But influenced less by these reports and the successful treatment of malaria than by the effects of quinin compounds in experimental trypanosomiasis, and by

the fact that certain peculiar biologic relationships (as bile solubility) have been found by Schilling[2] and Neufeld[3] to exist between trypanosomes and spirilla on the one hand and pneumococci on the other, Morgenroth and Levy[4] chose quinin and its derivatives as a basis and lead for chemotherapeutic studies in experimental pneumococcus infections. Best results were observed with optochin (ethylhydrocuprein) a synthetic derivative of hydroquinin (methylhydrocuprein) which latter exists in the cinchona bark or can be prepared synthetically. In experimental infections of mice the new drug was found to prevent the development of infection in some 90 per cent. of animals in which treatment was given after inoculation. Later, Guttman,[5] Morgenroth and Kaufman,[6] and Levy[7] reported further experiments indicating the protective and curative action of ethylhydrocuprein (base) and its hydrochlorid in experimental pneumococcus infections in mice.

While optochin (meaning the "best cinchonin") and its soluble salt, the hydrochlorid of ethylhydrocuprein, now marketed under the name "numoquin," have been found highly destructive for pneumococci *in vitro* and of some curative activity in the treatment of mice infected with these organisms, yet both have failed to fulfill early expectations in the treatment of pneumococcus pneumonias of human beings; but yet they are not without great interest and some practical value and Morgenroth,[8] continuing his efforts, has more recently produced isooctylhydrocuprein ("vuzin") for the treatment of staphylococcus, streptococcus and gas bacillus infections. The cinchona derivatives have proved, therefore, worthy of close attention in relation to the chemotherapy of bacterial infections and in this chapter I shall largely confine my discussion to a consideration of their bacteriostatic and bactericidal activities *in vitro* and their toxicity and curative activity in experimentally produced infections.

Selective Bacteriostatic and Bactericidal Activity of Ethylhydrocuprein (Optochin) and Other Cinchona Derivatives in Vitro in Relation to Chemotherapy.—Morgenroth and Levy found that ethylhydrocuprein possessed a high degree of pneumococcidal activity *in vitro* and this observation has been amply confirmed, although, as would be expected, the pneumococcidal dilutions published by different investigators have varied greatly according to the technic employed. Wright and his associates[9] found that a dilution 1:400,000 in serum killed pneumococci and that its bactericidal activity was about half as efficient as in a menstrum of water; they used, however, a pipet method carrying but a very few organisms and the bactericidal dilutions were, therefore, rather high. But in relation to chemotherapy, the observation that ethylhydrocuprein preserves a high degree of pneumococcidal activity in serum is of considerable importance as it indicates the probability of maintaining some bactericidal activity when absorbed into the blood.

Other cinchona compounds are much less pneumococcidal *in vitro*. Russo,[10] for example, observed that ethylhydrocuprein hydrochlorid in 1:16,000 kills pneumococci in three hours at room temperature, while the bactericidal activities of the hydrochlorids of isoprophylhydrocuprein, isobutylhydrocuprein, isbamylhydrocuprein, quinin and hydroquinin were much less.

Furthermore, ethylhydrocuprein is far more bactericidal for pneumococci than for other organisms. Moore[11] observed that while this compound was pneumococcidal in dilutions from 1:20,000 to 1:10,000,000 for different serological strains of pneumococci, yet streptococci, staphylococci, *M. catarrhalis*, *B. coli*, *B. typhosus* and *B. friedländer*, were not killed in dilutions of 1:10,000 except in the case of one strain of streptococcus which was killed in 1:20,000. Quinin hydrochlorid was pneumococcidal in 1:5000 to 1:10,000 and bactericidal for these other organisms in 1:1000 to 1:5000 dilutions. The specificity of the bactericidal action of cinchona derivatives on pneumococci was also studied by Cohen, Heist and myself[12] by cross-testing their efficacy against *B. typhosus*

and *S. aureus*, while also testing the bactericidal activity of phenol, mercuric chlorid, arsenobenzol and other chemicals for pneumococci.

In a general way, the results observed with *B. typhosus* and *S. aureus* and all the quinin salts were similar; that is, the inhibiting dilution usually ranged from 1:1000 to 1:2000. The value of ethylhydrocuprein as a bactericide was from 200 to 400 times greater for the pneumococcus than for *B. typhosus* and the staphylococcus; the various quinin derivatives showed a bactericidal activity against the pneumococcus ranging from 5 to 50 times greater than against the other microorganisms.

Ethylhydrocuprein hydrochlorid and other quinin compounds act somewhat slowly on organisms including pneumococci, and demonstrate more clearly their specificity for the pneumococcus in bacteriostatic than in bactericidal tests. According to the calculated amounts of quinin contained in the various salts tested, there is no definite relation. apparent between the percentage of alkaloid present and the bacteriostatic power of the compound.

Pneumococcidal Activity of the Blood after the Administration of Ethylhydrocuprein and Quinin.—While ethylhydrocuprein and practically all cinchona derivatives preserve a high degree of pneumococcidal activity *in vitro* in a menstrum of serum, it is of special interest in relation to chemotherapy to note that the administration of these compounds to human beings and the lower animals appreciably increases the pneumococcidal activity of the blood. Wright[13] was first to observe the pneumococcidal action of the serum of human beings and mice treated with ethylhydrocuprein. He called attention to the negative results obtained with rabbits. Scott[14] and Boecker[15] likewise obtained negative results with ethylhydrocuprein in the treatment of pneumococcus infections in rabbits. Moore[16] in an extensive study of this subject observed that the serum of rabbits which have been previously treated with a single dose of ethylhydrocuprein (optochin) exerts a bactericidal action on, and, later inhibits the growth of pneumococci in the test-tube. In a later study by Moore and Chesny,[17] it was observed that when optochin hydrochlorid was given by mouth to patients suffering from acute lobar pneumonia in such amounts that they receive at least 0.024 gm. per kilogram of body weight per twenty-four hours, and when the size and spacing of the individual doses are adequately regulated, a specific pneumococcidal action appeared in their blood within a few hours, and could be maintained more or less constant for several days.

Julian Lewis[18] has also observed an increased bactericidal activity of the serum of rabbits treated with ethylhydrocuprein, but Weiss,[19] who also worked with rabbits, observed that no pneumococcidal action of rabbit serum could be demonstrated after a single large tolerated dose, after a single fatal dose, after repeated injections of tolerated doses either by oral or by intravenous routes of various cinchona derivatives including ethylhydrocuprein hydrochlorid, quinin and urea hydrochlorid, quinin dihydrobromid, and quinin chlorohydrosulphate. He quotes Scott as stating that the destructive power of the rabbit liver for quinin alkaloids is 10 times greater than that of the guinea pig or mouse. But I am not quite so sure of the correctness of these propositions in view of Moore's results and since Borow and I,[20] working with the Heist-Lacy method,[21] which we found much more sensitive and less susceptible to contamination than plating methods, observed that the administration of ethylhydrocuprein (optochin) hydrochlorid to rabbits by intravenous, subcutaneous and oral routes results in rendering the whole blood decidedly pneumococcidal. Similar but much less marked results followed the administration of other quinin compounds as the bisulphate, hydrochlorid and quinin and urea hydrochlorid. The bactericidal properties of the blood rapidly disappeared however, after the administration of these compounds had ceased.

Acquired Ethylhydrocuprein (Optochin) Fastness or Resistance of Pneumococci.—As stated by Moore and Chesney, this is a consideration of

importance in the treatment of lobar pneumonia with optochin, namely, the possibility that a dosage which is too small, apart from failing to confer a pneumococcidal action on the circulating blood, may give rise to conditions which favor the acquisition by the infecting pneumococci of a condition of resistance or fastness to the drug. Indeed Morgenroth and Kaufmann,[22] Tugendreich and Russo[23] and others have succeeded in demonstrating this possibility both *in vitro* and *in vivo* and therefore in the treatment of disease with optochin it would appear desirable to avoid such a low concentration of the drug in the blood as would permit the pneumococci to develop this property.

Mechanism of the Bacteriostatic and Bactericidal Activity of the Cinchona Derivatives.—Practically nothing has been yet discovered concerning the mechanism of the bactericidal and bactericidal action of ethylhydrocuprein and other cinchona derivatives for pneumococci and other organisms *in vitro*. Why these compounds possess a high degree of selective activity for pneumococci is likewise unknown. Something in their molecular structure probably establishes a special affinity for the protoplasm of these organisms, but how they act in producing genesistasis or bacteriostasis and bactericidal effects has not been elucidated. Very probably the mechanism is similar to that concerned in the destructive effects of quinin for the malarial protozoa and this subject is discussed with detail in Chapter XXXII. Here it may be stated, however, that while optochin (ethylhydrocuprein) is highly pneumococcidal, it is regarded as inferior in the destruction of malarial parasites indicating that it does not possess a pan-therapeutic activity, and that for the bacteria its chemical constitution renders it more especially destructive for the pneumococcus.

Chemistry of the Cinchona Compounds in Relation to the Chemotherapy of Bacterial Infections.—We may now briefly consider the very difficult and complex subject of the chemistry of ethylhydrocuprein (optochin) and other cinchona derivatives in relation to their toxicity and pneumococcidal activities. Naturally, attempts have been made to improve upon optochin, at least to reduce its organotropism or toxicity for the host, but so far with little or no success.

In the first place it may be stated that while the toxicity of salts of quinin bears some relation to their content of alkaloid (varying from 20 per cent. in the tannate to 81.8 per cent. in the hydrochlorid and 75 to 80 per cent. in ethylhydrocuprein), there is no constant relation between alkaloid content, bactericidal activity *in vitro* or protective and curative activity *in vivo*. The alkaloid itself is far inferior to ethylhydrocuprein in pneumococcicidal activity as is quinin hydrochlorid, which contains approximately as much of the alkaloid. In other words, the side chains of the molecule exerts a marked effect upon the biological properties of these compounds.

Morgenroth and Bumke[24] have stated that in their opinion the specific pneumococcicidal activity of ethylhydrocuprein is in the ethoxy-group, but that in spite of its remaining intact the bactericidal value decreases in changing ethylhydrocuprein to the corresponding ketone. Rosenthal[25] gives a good description of the chemistry of this compound, the chemical structure of optochin and other quinin compounds, including "vucin," and one of the best investigations upon the chemistry of these and new synthesized aromatic derivatives of cinchona is by Jacobs and Heidelberger.[26] Felton and Dougherty[27] have studied a number of their new compounds for bactericidal activity *in vitro*, toxicity and curative activity in infected mice and rabbits with interesting results showing a relationship between chemical constitution and biological effects.

Toxicity of Ethylhydrocuprein and Other Cinchona Derivatives in Relation to the Chemotherapy of Bacterial Diseases.—In Chapter XXX the toxicity of the ordinary salts of quinin is discussed in relation to the chemotherapy of malaria and at present I wish to briefly review the toxicity of the base optochin and its soluble salt the hydrochlorid of ethylhydrocuprein, in relation to the chemotherapy of bacterial diseases, since it is doubtless apparent that these compounds have commanded most attention in this connection although, as already stated, the ordinary salts of quinin have been employed by Cohen and other clinicians in the treatment of pneumococcus pneumonia for many years.

Toxicity for the Lower Animals.—Moore[28] found that the highest tolerated dose of ethylhydrocuprein for rabbits by subcutaneous injection was about 0.1 gm. per kilogram of weight and 0.02 to 0.05 gm. by intravenous injection. Optochin base suspended in oil was tolerated in about 0.125 gm. per kilogram by subcutaneous injection and 0.075 gm. by intramuscular administration. As stated by Moore, ethylhydrocuprein in a toxic dosage gives rise to certain characteristic symptoms in rabbits, the variety and intensity of which depend in part on the dose, in part on the route by which it is given, and in part on the location of the injection.

Smith and Fantus[29] have reported that the minimal lethal dose of ethylhydrocuprein for mice is 0.5 mg. per gram of body weight; the dose for quinin is given as slightly higher or 0.7 mg. per gram of body weight. They found that optochin will reduce blood-pressure more than quinin and cause quicker cardiac death. Hunt[30] found the lethal dose of hydroquinin to be from 0.35 to 0.33 mg. per gram, and this was confirmed by Morgenroth and Halberstadter. Webb[31] observed that the minimal fatal dose of quinin sulphate and dihydrochlorid in 1:8 dilution for monkeys by subcutaneous injection was about 0.2 to 0.25 gm. per kilogram of weight. Cohen, Heist and I[32] found the lethal and maximum tolerated doses of ethylhydrocuprein hydrochlorid and some of the salts of quinin approximately as follows per kilo of weight:

Compounds	Lethal Dose		Tolerated Dose		
	Intraven.	Intramus.	Intraven.	Intramus.	Subcut.
Ethylhydrocuprein hydrochlorid	0.06	0.5–0.6	0.02–0.04	0.03–0.07	0.08–0.1
Quinin and urea hydrochlorid...	0.05–0.06	0.5	0.02–0.07	0.03–0.08	0.09–0.15
Hydroquinin hydrochlorid.. ...	0.04–0.05	0.06 0.25	0.02–0.06	0.02–0.06	0.08–0.1
Quinin hydrochlorid.....	0.5–0.06	0.5–0.6	0.02–0.05	0.03–0.07	0.09–0.18

As a general rule, mice withstood slightly larger doses than rabbits. Individual experiments also indicated that young rabbits were more resistant than older and heavier animals, confirming the observations of Moore in this respect. With the assistance of Idsumi and Sands, I have also determined the maximum tolerated dose of ethylhydrocuprein for white rats and rabbits by intraperitoneal, intrapleural and subthecal injections[33] with these results: (a) subcutaneous injection, 0.5 to 0.55 gm. per kilo; (b) intraperitoneal injection, 1.15 gm.; (c) intrapleural injection, 0.02 to 0.04 gm.; (d) intravenous injection, 0.03 to 0.04 gm. and (e) intraspinal injection, 0.0003 to 0.0005 gm. per kilo. By intracisternal or intraventricular injection the dose is slightly less being 0.0005 to 0.0001 gm. per kilo for normal animals and about 0.0003 gm. per kilo by repeated injection for animals with acute meningitis.

Toxicity of Ethylhydrocuprein (Optochin) for Human Beings.—A few years ago ethylhydrocuprein was rather widely employed in the treatment of lobar pneumonia and toxic effects were sometimes produced. Moore[34] has studied these with particular care and stated in 1917 that only one death had been, in part, ascribed to the drug by Smith and Fantus;[35] even in this case it is not entirely clear that heart failure was occasioned by the optochin. The toxic symptoms noticed have generally been transient and have usually disappeared when the drug was discontinued. The principal ones that occur are referable to the eye and the ear. In some cases vomiting has been reported; this may be due in some instances to the bitter taste of the drug, which rivals quinin in that respect. Vomiting, however, is rather infrequent, although one disadvantage connected with it is the possibility that the drug may be ejected. In only one of Moore's cases was vomiting frequent enough to interfere with the administration of the drug and in that case it is doubtful if the optochin was the cause.

The symptoms referable to the ear comprise ringing and roaring noises and partial deafness. In six of his cases tinnitus and partial deafness were associated, and in one case there was tinnitus alone, without deafness. In every case these symptoms were transient, disappearing shortly after the drug was discontinued. Indeed, in some instances these symptoms disappeared or became less evident while the drug was still being given in full amounts.

Toxic Amblyopia in Human Beings and Rabbits.—As stated by Moore, the symptoms referable to the eyes are more serious. It is difficult to determine the percentage of recorded instances of eye trouble due to the use of the drug; a review of the literature would seem to show that about 4.57 per cent. of all reported treated patients have had transient amblyopia or amaurosis. In one recorded case has there been permanent, almost complete, blindness. In this case, reported by Oliver, the patient received 5 grains (0.3 gm.) every three hours, which would mean 2.4 gm. per twenty-four hours, a dose greater than that usually given. In all other cases of amblyopia and amaurosis reported in the literature, the visual disturbances apparently disappeared after the drug had been discontinued.

With the impairment of vision the pupils may be widened, may not react to light, and ophthalmoscopic examination may show tortuosity of the retinal vessels, and generally shows narrowing of the arteries. Whether these changes in the pupils and vessels precede the impairment of vision for any considerable time cannot be stated; they did not appear in any of Moore's cases with the exception of the one that had already developed amaurosis, although all of the patients who received the drug had daily ophthalmoscopic examinations. Optochin amblyopia is said to resemble closely that of quinin amblyopia.

There is some ground for believing that the visual disturbances may depend to a certain extent on idiosyncrasy. That a condition of susceptibility to the drug may be an important factor is indicated by the fact that some patients develop amblyopia after they have had only 2 gm. of less of optochin, whereas others do not acquire it even after they have received as much as from 10 to 16 gm. A study of the literature seems to indicate, however, that the more the daily dosage is subdivided, the less frequent are the visual disturbances. If subsequent observation bears out this point, an additional reason is supplied for not giving the drug in three large doses in twenty-four hours, which method, as shown previously, probably gives rise to considerable fluctuations in the concentration of optochin in the circulating blood. It is possible, also, that the maintenance of the body-weight relation-

ship may help to diminish the frequency of occurrence of this complication by preventing overdosage. Daily examination of the eyegrounds and the pupils may give warning of impending symptoms and thus indicate the discontinuance of the drug, or reduction of dosage.

About three years ago I had one of my students, the late Dr. Andrew L. Forster, investigate the toxic effects of quinin and urea hydrochlorid and ethylhydrocuprein hydrochlorid upon the eyes of white rabbits. Two groups of animals were employed, the drugs being administered by ingestion, subcutaneous and intravenous injection. I was surprised to learn of the really enormous total dosage required to produce toxic effects and surmise that the eyes of human beings are far more susceptible than those of rabbits, and especially to optochin (probably less so to quinin and urea hydrochlorid and other ordinary salts of quinin).

Dr. Forster summarized his study with the statement that rabbits were able to tolerate enormous doses of quinin and urea hydrochlorid and ethylhydrocuprein hydrochlorid without showing any special signs of intoxication. The first group received 238 doses, or a total of 5.55 gms. within a period of three months. This tolerance was demonstrated by the fact that all rabbits, those receiving the largest as well as those receiving the smallest doses, apparently enjoyed the same health and retained their same approximate weights.

The second group received much larger total amounts of the drugs within a comparatively short period, namely, 49 doses within four weeks or a total of 2.96 gms. The reason for this increased amount was to reach the toxic dose which appeared to be approximately 0.080 gm. per kilo given hourly for two to four doses.

It is noteworthy that during the entire period of the experiments none of the rabbits ever exhibited any corneal anesthesia, dilatation of the pupils, ischemia of the retina or constriction of the retinal arteries, evidence of visual disturbance or any other ophthalmoscopic changes in the fundus. Microscopically the first group showed perivascular infiltration of mononuclear and plasma cells in the cerebrum in three cases, and to a less degree in one case in the cerebellum. In all other cases, the pons, cerebellum and optic nerves were negative. In the retina the ganglion cell layer in all cases showed marked changes, such as vacuolization of the protoplasm and nuclei, the breaking up of the chromatin, the loss of the nuclear wall and the extrusion of its contents, the loss in staining properties, a decrease in the number of ganglion cells and the appearance of hyaline-like bodies.

Ethylhydrocuprein (Optochin) and Other Cinchona Derivatives in the Chemotherapy of Experimental Trypanosomiasis and Bacterial Diseases. With this review of the toxicity, bacteriostatic and bactericidal activities of ethylhydrocuprein (optochin) and other cinchona derivatives, we may now consider their protective and curative effects in experimental bacterial infections and especially those produced by virulent pneumococci. After all, these effects are of most interest and importance because so many factors may reduce the curative value of chemical agents displaying an extremely high selective bacteriostasis in the test-tube, as that of ethylhydrocuprein for the pneumococcus.

Ethylhydrocuprein in the Treatment of Experimental Trypanosomiasis.—I have already mentioned that Morgenroth was influenced in selecting the cinchonics as a "lead" in this work by reason of their influence upon trypanosomes and it may be of interest to first state in passing that in trypanocidal tests employing white rats infected with *T. equiperdum* and treated with intravenous injections of ethylhydrocuprein, only the maximum tolerated

dose of this drug, namely, 0.030 to 0.060 gm. per kilogram of weight, retarded the development of trypanosomiasis for 24 to 48 hours, but never succeeded in sterilizing the animals; similar results were observed with quinin bisulphate and quinin dihydrochlorid in dose of 0.060 gm. per kilogram.

Morgenroth and Tugendreich,[36] however, observed that while ethylhydrocuprein itself may fail, a combination with sodium salicylicate sometimes succeeded, while a combination of quinin with the salicylate failed. As would be expected, best results were secured by a combination of ethylhydrocuprein with salvarsan (arsphenamin) in dose of 0.0015 to 0.003 gm. per kilogram of weight.

Ethylhydrocuprein (Optochin) and Other Cinchona Derivatives in the Chemotherapy of Acute Pneumococcus Bacteremia of the Lower Animals.—When virulent pneumococci are inoculated intraperitoneally or subcutaneously in white mice, rapidly developing and fatal bacteremia develops because of the extreme susceptibility of these animals to this organism. Patches of pneumonia may be produced by injecting the organisms into the lungs directly through the chest wall, but the resulting fatal bacteremia constitutes the chief lesion.

Morgenroth and Levy originally employed mice infected with pneumococci and treated them with subcutaneous injections of optochin (base) suspended in oil. With this method 80 to 100 per cent. of mice were protected against fatal infections and the specific action of ethylhydrocuprein on the pneumococcus was found to be greater than that of any other compound of the series examined, namely, quinin, hydrocuprein and isoamylhydrocuprein. Similar results were reported by Morgenroth and Gutman [37] and confirmed by Gutman[38] working with mice and by Engiver[39] working with guinea-pigs. Levy[40] also found that mice infected with *Streptococcus mucosus*, which I believe was probably a Type III pneumococcus, could be saved against fatal bacteremia by four daily subcutaneous injections of 2-per cent. suspensions of optochin in oil in dose of 0.4 c.c. per 20 grains of weight. Moore[41] has also confirmed the curative effects of optochin base in pneumococcus infection of mice. Cohen, Heist and I,[42] however, working with the soluble ethylhydrocuprein hydrochlorid were not able to elicit as good results as reported by these investigators. As a general rule, when the drug in dose of 0.02 gm. for mice and 0.01 gm. for rabbits per kilo and the pneumococci were injected within a few seconds of each other, the life of about 20 per cent. of the animals was prolonged indefinitely, while in the balance life was prolonged but a few days beyond that of the controls. Both hydroquinin hydrochlorid and quinin and urea hydrochlorid sometimes showed slight protective value.

Further experimentation may improve the chemotherapeutic value of ethylhydrocuprein by additions, substitutions, or other molecular modifications but in my opinion optochin stands today in a position somewhat similar to that of atoxyl in the treatment of experimental trypanosomiasis. Lewis[43] and others have likewise failed to demonstrate as high curative activity of ethylhydrocuprein, in rabbits at least, but mixtures of the drug with antipneumococcus serum have yielded even better results, and it may be profitable to now review this phase of the subject.

Chemoserotherapy of Experimental Pneumococcus Infections.—Neufeld and Engwer[44] and Engwer[45] working with the pneumonias of guinea-pigs produced by the direct intrapulmonary injection of pneumococci through the chest wall according to the method of Neufeld and Ungermann, observed that the curative effects of ethylhydrocuprein were greatly enhanced by administering antipneumococcus serum at the same time. Boeknke,[46]

working with mice, stated that optochin alone protected 20 per cent. of the infected animals, serum therapy alone about 33 per cent., while a combination of the two protected as high as 90 per cent.

Observing that antipneumococcus serum for Type II pneumococci was less efficient than the serum for Type I, Moore[47] studied the influence of a combined optochin and serum treatment for Type II infections among mice and found that a single dose of optochin suspended in oil and injected subcutaneously, which by itself has practically no protective effect, was capable of increasing the threshold value of Type II serum at least 50 times, and that this effect was proportionately many times greater than a simple summation of the protective and curative effects of the two substances separately. Steinfield and I[48] also observed that ethylhydrocuprein hydrochlorid by subcutaneous injection in doses without protective value, usually increase the protective value of antipneumococcus serum Type I in a slight but definite manner in severe infections of mice and rats with homologous pneumococci. Several of the commoner compounds of quinin, as quinin and urea hydrochlorid, quinin bromid, and quininchlorohydrosulphat, given subcutaneously in doses without any appreciable influence on severe and fatal pneumococcus infections, occasionally increase the protective power of antipneumococcus serum but to a lesser extent and less regularly than ethylhydrocuprein hydrochlorid.

Ethylhydrocuprein in the Chemotherapy of Experimental Pneumococcus Meningitis.—Owing to the highly fatal character of pneumococcus meningitis and the inadequacy of methods of treatment, Idzumi and I[49] undertook a study of the effects of ethylhydrocuprein in experimental meningitis of rabbits, since systematic studies in the chemotherapy of pneumococcus meningitis do not appear to have been made. Owing to the comparative ease with which the disease may be produced experimentally, provided highly virulent cultures are available, it would appear a particularly interesting field for experimental study under conditions closely resembling a disease occurring among human beings.

Small doses like 0.001 gm. in 0.5 c.c. of salt solution per kilo of body weight usually did not produce symptoms, but sometimes produced slight congestion of the meninges at the site of the injection; 0.0005 gm., equivalent to 0.5 c.c. of a 1:1000 dilution per kilo, did not produce any symptoms or discernible changes in the meninges and was generally employed by us in the therapeutic experiments. This dose corresponds to 30 c.c. of a 1:1000 dilution per 60 kilos of weight and represents a safe dose of ethylhydrocuprein hydrochlorid for adult persons by intraspinal administration.

Therapeutic tests were now conducted with the general result that the subthecal injection of single doses of ethylhydrocuprein, in amounts of 0.5 c.c. of 1:500 and 1:1000 solution per kilo of body weight, had a distinct beneficial effect on the course of experimental meningitis in rabbits produced by a Type I pneumococcus of moderate virulence, when administered not later than 4 to 6 hours after the injection of organisms; when given 24 hours after the pneumococci this effect was not apparent. With a meningitis produced by a more virulent culture of Type II pneumococci, single doses of this drug had but slight or no effect on the duration of the lives of the experimental animals, but in experiments consisting in the repeated intraspinal injection of smaller doses of pneumococci followed in a few hours by ethylhydrocuprein, the protective and curative effects of the drug were more apparent. However, mixtures of specific antipneumococcus serum and ethylhydrocuprein did not yield results in the treatment of experimental meningitis produced by Type I pneumococci, superior to those observed

with the drug alone and the serum alone was without appreciable effect on the course of the experimental lesions. Similar results have been observed more recently on the treatment of acute pneumococcus meningitis of dogs[50] treated with ethylhydrocuprein (numoquin) by intravenous, intracisternal and intraventricular injection. In these the drug alone has been without appreciable effect unless the subarachnoid space was first washed out with saline or Ringer's solution. I have treated eleven cases of pneumococcus meningitis of otitic or nasal origin with the compound by spinal puncture and the daily or twice daily intraspinal injection of 15 to 30 c.c. of 1:1000 solution without a single recovery.

Lamar,[51] having shown that sodium oleate in 0.5 to 1 per cent. solution is destructive for pneumococci and that weaker solutions as 0.1 to 0.001 per cent. lessen the virulence of pneumococci and increase their susceptibility to the destructive action of antipneumococcus serum, boric acid overcoming the inhibitory influence of the protein constituents, we included a study of these substances alone and in combination with ethylhydrocuprein on the pneumococcus in rabbits with meningitis, to determine whether sodium oleate increases the susceptibility of virulent pneumococci to the destructive activity of ethylhdyrocuprein. Intraspinal injection of these mixtures appeared to prolong the lives of some infected animals and not of others, as compared with animals receiving ethylhydrocuprein alone. While sodium oleate undoubtedly reduces the vitality of pneumococci or at least increases their susceptibility to the destructive influences of ethylhydrocuprein when the organisms are exposed to the oleate *in vitro*, our results in the treatment of experimental pneumococcus meningitis in rabbits with mixtures of ethylhydrocuprein and sodium oleate have not yielded the encouraging results observed by Lamar in the treatment of experimental meningitis in monkeys with mixtures of specific antipneumococcus serum, boric acid and sodium oleate, and in our experiments the mixtures did not prove superior to those observed with only ethylhydrocuprein.

Ethylhydrocuprein, Sodium Oleate and Bile in the Chemotherapy of Experimental Pneumococcus Pleuritis.—Since these experiments on pneumococcus meningitis have indicated that it may be possible to inject enough ethylhydrocuprein into closed sacs to raise the pneumococcidal activity of the exudates without danger of irritation of the serous membranes or the absorption of toxic amounts, Sands and I[52] have conducted similar experiments on acute suppurative pneumococcus pleuritis induced in guinea-pigs and dogs as having a possible value in the treatment of pneumococcus pleuritis with empyema developing during croupous pneumonia. The results have indicated that in the treatment of pneumococcus meningitis and pleuritis, sufficient amounts of ethylhydrocuprein may be injected intrathecaly or intrapleurally to exert considerable pneumococcidal activities without irritating these membranes or causing toxic effects. When the drug is administered by mouth, intramuscularly, or intravenously, it may be so highly diluted in the blood that enough cannot be administered to raise the pneumococcidal activity of the blood sufficiently for pronounced curative effects without danger of toxicity, but in the subarachnoid and pleural sacs dilution is by spinal fluid or exudate alone and is much less than in the blood.

The experimental lesions in guinea-pigs were very severe, consisting of acute suppurative pleuritis which involved both pleurae and the external portions of the pericardium. The intrapleural administration of ethylhydrocuprein in a dose corresponding to about 2 c.c. of a 1:500 dilution per kilo of weight in each cavity, a total of 0.008 gm. of drug, had a marked curative influence in the majority of instances unless treatment was delayed longer

than 24 hours. Such a delay permitted the development of severe bilateral pleuritis, pericarditis, and pneumococcus bacteremia. Multiple injections of 1 c.c. of a 1:500 solution per kilo into each pleural cavity had an even more pronounced effect upon less severe infections. These results indicate the possibility of chemical disinfection of infected serous cavities with drugs showing a marked and selective bactericidal activity for the infecting bacterium in the presence of pus, as optochin or ethylhydrocuprein hydrochlorid for the pneumococcus, even though sufficient amounts of the drug cannot be administered by mouth, intramuscularly, or intravenously to influence a localized inflammatory process.

Vucin in the Chemotherapy of Experimental Stapylococcus and Streptococcus Infection.—As previously stated, "vucin" or isocytylhydrocuprein, prepared by Morgenroth,[53] has been found highly bactericidal for staphylococci and streptococci and has been successfully employed in 1:2000 solution for the treatment of gas bacillus infection of wounds. Stronger solutions, however, are necrotizing. Solutions as high as 1:12,000 in dose of 1 c.c. per 20 grams have been found curative for mice inoculated subcutaneously with virulent streptococci by Morgenroth's method and similar results have been observed in staphylococcus infections. I have had no experience with the compound and according to Heffter,[54] the Remedy Committee of the German Society for Internal Medicine have stated that its use is still in the experimental stage.

Mechanism of the Curative Activity of Ethylhydrocuprein (Optochin) and Other Cinchona Derivatives in Pneumococcus Infections.—As previously stated in discussing the mechanism of the pneumococcidal activity of the cinchona derivatives *in vitro*, we do not know just how they destroy organisms, unless it is by a mechanism similar to that concerned in the destruction of the malarial protozoa described in Chapter XXXII. *In vivo* the same mechanism is doubtless accountable, as ethylhydrocuprein (optochin) undoubtedly possesses a high degree of bactericidal activity for pneumococci in the blood and exudates even though it may fail to reach and destroy appreciable numbers of the organisms in the lesions of lobar pneumonia. The results of the experiments discussed above indicate that these compounds may enhance immunological destruction of pneumococci by reducing the resistance of the organisms to the bactericidal activity of the blood.

Does ethylhydrocuprein and other cinchonics tend to increase antibody production for the pneumococcus? As far as I am aware, no one has studied this problem sufficiently well to warrant an opinion, but I doubt that any such mechanism is involved. It may be, however, that ethylhydrocuprein favors the phagocytosis of pneumococci by lowering the resistance offered by the organisms and it would appear abundantly proven that phagocytosis plays an important rôle in the recovery from pneumonia and other pneumococcus infections.

Steinfield and I,[55] have found that *in vitro*, weak solutions of ethylhydrocuprein and other cinchona derivatives materially increase the phagocytosis of virulent pneumococci. In our experiments it was observed that pneumococci possessing some degree of virulence for rabbits and mice may undergo spontaneous phagocytosis by the exudative leukocytes of rabbits, but that ethylhydrocuprein hydrochlorid, quinin and urea hydrochlorid, and other compounds or derivatives of quinin in high dilutions accelerate the phagocytosis of pneumococci by rabbit leukocytes. Low dilutions of cinchona derivatives, however, were found to retard phagocytosis and induce degenerative changes in leukocytes.

The administration of ethylhydrocuprein hydrochlorid, quinin and urea hydrochlorid, and other quinin derivatives to rabbits by intramuscular injection was found to increase the phagocytic powers of the sera for pneumococci and it is probable that these quinin derivatives in high dilution accelerate phagocytosis by reducing the virulence and resistance of the pneumococci and also by stimulating the phagocytic activities of the leukocytes.

Furthermore, the intramuscular injection of ethylhydrocuprein hydrochlorid, quinin and urea hydrochlorid, and other cinchona derivatives in rats increases the total number of leukocytes in the peripheral blood while lesser degrees of leukocytosis follow the intravenous injection of these compounds.

These experiments indicate that probably a part of the curative effect of quinin and urea hydrochlorid, ethylhydrocuprein, and other cinchonics in pneumococcus infections is to be ascribed to their influence on phagocytosis, and I am inclined to attribute no small part of their curative effects *in vivo* to this mechanism in conjunction with a direct bactericidal action of the compounds.

Ethylhydrocuprein (Optochin) and Other Cinchona Derivatives in the Chemotherapy of Bacterial Diseases of Man.—In conclusion it may be stated that ethylhydrocuprein (optochin) and other cinchonics have been reported by many competent clinicians as possessing some practical therapeutic value in the treatment of pneumococcus bacteremia and pneumonia of human beings. It is true that early expectations have not been realized and that a *therapie magna sterilisans* has not been secured, but unquestionably it is possible by proper administration to raise the pneumococcidal activity of the blood and probably increase phagocytosis and these results are doubtless worth while, as I shall discuss with more detail in Chapter VIII.

Ethylhydrocuprein in 1-per cent. solution has also proven efficacious in the treatment of pneumococcus conjunctivitis and I believe it may possess some curative activity in the local treatment of pneumococcus sinusitis, mastoiditis, otitis media, as well as in pneumococcus meningitis, pleuritis and for the disinfection of the mouth in carriers.

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CHAPTER VII

COMPOUNDS OF MERCURY, ARSENIC AND OTHER SUBSTANCES IN THE CHEMOTHERAPY OF BACTERIAL DISEASES

Probably no class of chemical agents has commanded as long, as much or as hopeful an interest in the chemotherapy of bacterial diseases as the inorganic and organic salts of mercury. The very fact of the well-established curative activity of mercurial compounds in syphilis was of itself sufficient for focussing attention upon their possible curative effects in bacterial diseases and almost simultaneously with the earliest developments in bacteriology, was the discovery of the extremely high bactericidal activity *in vitro* of mercuric chlorid and other salts of mercury for various bacteria. Naturally enough these observations led to the expression of most hopeful expectations in the treatment of disease, although Koch[1] showed quite early that mercury was without apparent effect in the treatment of experimental tuberculosis of guinea-pigs, despite the fact that the vapors and various soluble inorganic salts were found to be highly tuberculocidal in the test-tube.

But mercury has always had a peculiar fascination for many physicians in the treatment of bacterial infections by local application and even for the treatment of systemic infections by ingestion, intramuscular or intravenous injection, as in the use of calomel or mercuric chlorid in the treatment of typhoid fever by attempted disinfection of the intestines, the injection of the

succinimid in the treatment of tuberculosis, etc. Of course it has been long realized that the high organotropism or toxicity of the mercurial compounds constituted a stumbling block to their usefulness as disinfectants of the tissues, but experimental chemotherapeutists have frequently considered the possibility of reducing their toxicity by the synthesis of new compounds without too greatly reducing parasiticidal effects. It is well known, of course, that mercury may be so firmly bound in a molecule, as in full-complex compounds, that the compound is eliminated without much dissociation and consequently with greatly reduced toxic effects; but I shall later discuss this phase of the subject, wishing merely at this time to express the opinion that the hopes of mercury in chemotherapy are probably justified and that it occupies an important place as a "lead" in the chemotherapy of bacterial diseases and particularly since mercurochrome was discovered and found so encouraging by Young, White and Schwartz[2] in the treatment of local and systemic bacterial infections. A great deal of time and effort have already been expended in chemotherapeutic studies with the mercurials and especially in relation to the treatment of syphilis, but it cannot be said that the possibilities have been exhausted and it is to be hoped that experts in this particular field of chemistry will continue their efforts, especially in relation to the treatment of bacterial diseases.

Naturally enough, the organic arsenicals and especially arsphenamin and neoarsphenamin have also been tried, particularly in a clinical or empirical manner in the treatment of some bacterial diseases, since they quickly proved so efficacious in the treatment of spirochetic infections and since some biologists regard the organisms as closely related to the bacteria. But next to mercury probably the salts of silver have commanded most attention, and especially various organic compounds for the treatment of bacterial infections by local application. The salts of zinc have also been employed, the chlorid having been selected by Lister for this purpose in his pioneer work on the antiseptic treatment of wounds. Compounds of gold, copper, bismuth and some of the rarer metals have also been tried in experimental tuberculosis. Indeed, an extensive literature has accumulated upon these subjects, but it is hardly demanded that more than a fraction of it be reviewed or considered in this chapter in relation to the chemotherapy of bacterial infections in the modern sense.

Bacteriostatic and Bactericidal Activity of Compounds of Mercury, Silver, Arsenic, Tin, Zinc and Bismuth in Relation to Chemotherapy.—For example, a great deal of investigation has been devoted to a study of the bactericidal and bacteriostatic properties *in vitro* of a host of inorganic and organic compounds of mercury, arsenic, gold, copper, etc. The results are not without a certain value in relation to chemotherapy, but only in a broad and general manner, since it is well established that, in the presence of blood, exudates, etc., as encountered *in vivo*, bactericidal effects may be greatly reduced and that the organotropism or toxicity of the compounds for body cells may be sufficiently high to prevent their use in the practical treatment of bacterial diseases and especially systemic infections by intravenous administration of the compounds. Furthermore, the bacteriostatic and bactericidal properties described by different investigators are so greatly influenced by the technic employed and especially the menstrum, that only broad and general comparisons and deductions are warranted; likewise, the results vary according with the organism employed in the tests, and indeed, with different strains of the same organism, as I shall shortly discuss in a consideration of the important subject of selective bacteriostasis exhibited by mercury and other metals.

Without doubt the soluble salts of *mercury* are among the most potent, if not the most potent, known bacteriostatic and bactericidal agents *in vitro* in a menstrum of distilled water or simple saline solution. Even in a menstrum of blood, serum, pus or muscle extract, the majoriy of mercurial compounds maintain fairly high bactericidal values, so that they have long held the attention of chemotherapeutists in relation to the possibility of synthesizing useful compounds for the treatment of local and systemic bacterial infections.

It is hardly necessary, however, to pause here and discuss with much detail the bactericidal effects *in vitro* in water or saline solution of the salts of the heavy metals, because these effects are of value only in relation to the sterilization of instruments and other objects; in relation to the chemotherapy of localized and superficial infections of the skin, mucous membranes and wounds by local application or of systemic infections by subcutaneous or intravenous injection, as well as the disinfection of sputum, feces, urine, pus, etc., only the effects produced *in vitro* in the presence of blood, serum, or tissue extractives possess interest and practical importance. Possibly an exception may be made to this general statement in dealing with chemical agents primarily designed for treatment of localized infections of the urinary system in which useful tests *in vitro* may be conducted with a menstrum of sterile urine, as employed by Young, White and Swartz in their studies with mercurochrome.

I have selected a few compounds of the heavy metals for purposes of illustration in the present discussion because of their wide clinical use in the treatment of localized infections of mucous membranes and because in a general manner they serve as examples of the comparative bactericidal activities of the heavy metals represented by them. Working with the blood serum—muscle extract medium seeded with staphylococci and conducting duplicate tests in exactly the same manner and at the same time, except that sterile distilled water was substituted for this special medium, I have found that the *final* concentrations proving completely bactericidal at the end of twenty-four hours at 38°C. were approximately as follows:

Substances	Serum-muscle Medium	Water
Mercuric chlorid	1:1,600	1:1,250,000
Mercurophen	1:1,800	1:2,500,000
Mercurochrome	1: 200	1: 125,000
Silver nitrate	1: 200	1: 640,000
Argyrol	1: 20	1: 1,500
Protargol	1: 8	1: 1,500
Zinc chlorid	1: 80	1: 500
Arsphenamin (Disodium)	1: 60	1: 1,500
Neoarsphenamin	1: 20	1: 1,000
Dakin's solution	1: 8	1: 800
Boric acid	Not in 1: 40	1: 3,000

In other words, mercurophen or sodium oxy-mercuri-ortho-nitro phenolate, first described by Hantzsch and Auld[3] under the name of "merkurinitrophenole" but independently rediscovered and first biologically tested by Schamberg, Raiziss and myself,[4] is about twice as bactericidal for staphylococci and various other organisms in this medium as mercuric chlorid, while mercurochrome is about 8 times, silver nitrate about 8 times, argyrol about 80 times, protargol about 200 times, and zinc chlorid about 20 times, less bactericidal. It will be noted, however, that in a menstrum of distilled water the bactericidal values were all higher, but varying among the different compounds. Thus the bactericidal activity of mercurophen and mercurochrome was greatly reduced, but proportionately less in the blood-muscle extract medium than the other compounds tested, and they also produced less or no precipitation with the proteins and salts (phosphates) of the medium, which constitutes an advantageous property from the standpoint of chemo-

therapy. With a medium of sterile urine, Young and his associates observed that, whereas the silver proteins like argyrol and protargol in 1:1000 failed to kill *B. coli* or *Staphylococcus aureus* in one hour, mercurochrome killed both organisms in about one minute.

Examples of this kind could be multiplied at great length but with little advantage, since it is so well established that bactericidal activity of chemical agents *in vitro*, even in the presence of proteins, are of limited value in relation to chemotherapy, although of great value in relation to the sterilization of instruments and the disinfection of discharges and excreta. Thus the bactericidal activity of boric acid is a great deal less than that of the mercurials, but yet it constitutes a valuable agent for the treatment of local infections, as discussed in Chapter VIII.

From the standpoint of the treatment of systemic bacterial infections by intravenous administration of medicaments, the salts of only two of the heavy metals are of interest and importance, namely, mercurochrome and arsphenamin. It is true that mercuric chlorid and other inorganic and organic salts have been used from time to time, but none have produced the rather encouraging results observed during the past two years with mercurochrome. This compound is much less bactericidal *in vitro* than mercuric chlorid, mercurophen and many other compounds in both blood and saline media, probably at least 50 times less bactericidal for such organisms as the staphylococci and streptococci, because it undergoes dissociation so slowly, but this property also accounts for its greatly reduced toxicity *in vivo* which permits of the administration of relatively large amounts.

The bactericidal activity of *arsphenamin* and *neoarsphenamin in vitro* is discussed at more length elsewhere, but I may state here that contrary to popular impressions, they are not without some degree of bactericidal activity *in vitro* in both blood and saline media. But their bactericidal properties are relatively feeble, being at least 75 times less than that of mercuric chlorid.

But few of the salts of other heavy metals have been administered intravenously on a large scale for the treatment of systemic infections and their bactericidal effects *in vitro* are of chief interest only in relation to the treatment of localized infections. *Silver* compounds have been employed in some instances, but not on a large scale because of their toxicity and the danger of producing argyria. *Tin* and especially a mixture of chemically pure tin oxid and metallic tin known as "stannoxyl" has been advocated for the treatment of staphylococcus infections, but in my experience these compounds are but feebly bactericidal *in vitro;* 1:100 solutions or suspensions of stannous chlorid, stannic chlorid and stannic iodid in sterile water may kill *staphylococcus aureus* in less than five minutes but stannoxyl failed to do so in 60 minutes. In 1:1000 concentration all of these preparations failed to kill staphylococci in 60 minutes except a suspension of stannous chlorid which was bactericidal in 15 minutes. The tin preparations, therefore, do not command much interest from the standpoint of bactericidal activity *in vitro*.

The same may be stated of the *zinc* salts, although they have long been known to have antiseptic properties and the chlorid especially has been used by Lister, Kocher, and others. Its bactericidal activity, however, is far inferior to that of most mercury or silver salts.

Dakin and Dunham state that "the salts of *bismuth* do not appear to have been examined very carefully as regards their bactericidal action, although their action in checking undesirable fermentations in beer worts was discovered long ago. Most normal bismuth salts are more or less rapidly decomposed by water with formation of insoluble basic salts, and some of these have found effective use as mild antiseptics capable of slow but prolonged action."

Rapidity of Bactericidal Activity of Mercury and Other Metals.—But the matter of rapidity of disinfection by compounds of the metals is deserving of mention and especially when they are employed for the treatment of localized bacterial infections. For example, in the treatment of urethritis, conjunctivitis, gingivitis, etc., the disinfecting agent is in contact with the tissue for probably only a matter of a few seconds and only those agents known to act quickly are apt to prove bactericidal under these conditions. Of course, rapidity of sterilization bears a relation to concentration of the chemical agent and its absorption or fixation by cells, but comparisons may be made by working with similar concentrations under identical conditions. Mercurophen, for example, acts much more quickly than mercuric chlorid. Chick[5] found that *B. paratyphosus* could withstand the action of five per cent. bichlorid for four minutes, and *Staphylococcus aureus*, for fifteen minutes, if at once treated with a potassium sulphid antidote. Of course, the rapidity of disinfection is greatly reduced in a medium of blood, serum, muscle extract, etc.; under these conditions organisms may survive 1:3000 mercuric chlorid for several hours and, while numerous additional examples may be cited, the above will probably serve my present purpose which is merely to state that rapidity of disinfection in the presence of pus, blood, secretions, etc., bears an important and practical relation to the worthiness of any chemical agent proposed for the treatment of localized bacterial infections by topical application.

Acquired Resistance of Bacteria to Compounds of the Metals.—The subject of acquired resistance of bacteria to compounds of mercury and other metals has not commanded much attention. But it is not without considerable academic as well as practical interest, and especially in relation to the treatment of urethritis and other localized infections by the long continued application of the silver, zinc and other compounds.

Spiess and Feldt, in their series of studies upon the effects of gold compounds in tuberculosis, observed that tubercle bacilli may acquire resistance or fastness to gold cyanid *in vitro;* Shiga, however, found that tubercle bacilli do not develop a tolerance for gold and DeWitt and Sherman state that, in their experiments, tolerance developed so slowly as not to be an important factor in the treatment of tuberculosis in guinea-pigs with gold.

I have experimented with mercurial compounds and cultures of *Staphylococcus aureus* and *B. coli* with the general result that after a prolonged series of transfers the organisms could be rendered distinctly more resistant and the same is probably true of compounds of the heavy metals and most bacteria in general; but results *in vitro* are obtained only after several months of patient work and I doubt if bacteria, like the gonococcus, acquire sufficient resistance *in vivo* to the silver protein and other bactericidal agents materially to reduce the bactericidal effects of these compounds.

Selective Bacteriostatic Activity of Mercurial, Silver and Other Organic and Inorganic Compounds.—As stated in preceding chapters, many of the anilin dyes display a selective bacteriostasis and bactericidal activity *in vitro* and ethylhydrocuprein (optochin) and other cinchona derivatives are known to be especially destructive for pneumococci; the question naturally arises, do any of the organic and inorganic compounds of mercury and other heavy metals display a similar selective bacteriostosis *in vitro?* This question is one of considerable theoretical and some practical importance, since an ideal compound would be one possessing a wide range of pan-therapeutic activity suitable for the treatment of diseases produced by different organisms.

Selective Bacteriostasis of Mercurial Compounds.—Insofar as the ordinary organic and inorganic *mercurial compounds* are concerned, it is my observa-

tion that they do not exhibit any noteworthy evidences of selective bactericidal activities, except that, as a class, they are slightly more effective against Gram-positive than against Gram-negative organisms. But nearly all the organic and inorganic salts possess a high degree of bactericidal activity for practically all bacteria and some, like mercurochrome, for example, have been found to influence *B. coli* infections as well as those produced by staphylococci, streptococci and gonococci. It is true that some organisms are more readily and rapidly killed *in vitro* than others, but these variations are readily explained by factors other than selective bacteriostasis. But mercurophen, however, does display a certain degree of selective activity for the Gram-positive cocci (staphylococci, pneumococci and streptococci) and is likewise somewhat more active for Gram-positive bacilli (anthrax, subtilis, diphtheria and tubercle bacilli), but this selective bacteriostasis is not so specific or marked as shown by gentian-violet for Gram-positive organisms. All of the mercurials, and especially mercurophen, are likewise particularly bacteriostatic for spores like those of *B. anthracis* and *B. subtilis*. Mercurochrome is also somewhat more bactericidal for the Gram-positive staphylococcus than for the Gram-negative colon bacillus, and metaphen (4-nitro-3, 5-bisacetoxymercuri-2-cresol), recently described by Raiziss and Severac,[6] has similar properties. I may also mention that mercurophen and metaphen do not precipitate the dissolved proteins in blood and pus, do not form amalgams with ordinary surgical instruments and constitute very useful bactericides for the disinfection of sputum, urine, feces, pus, etc., being particularly valuable for the rapid sterilization of instruments.

It may be stated, therefore, that while all of the mercurials possess some degree of selective bacteriostasis for staphylococci and other Gram-positive cocci, this property is especially well marked with mercurophen and metaphen and to some extent with mercurochrome. DeWitt[7] has observed that these compounds also display a high bactericidal activity for tubercle bacilli.

DeWitt[8] has also studied the inhibitory effects of a large number of other new organic compounds of mercury which lead to some interesting deductions on the chemistry of these substances in relation to tuberculocidal effects, to which I shall shortly refer in more detail.

Selective Bacteriostasis of Silver Compounds.—The silver salts, however, appear to be somewhat more selective for the Gram-negative cocci and especially the gonococcus, as recently observed by Hamilton,[9] with silvol, neosilvol and similar metallic silver colloids working with a modification of the U. S. Hygienic Laboratory method. According to Chick, the action of silver nitrate on *B. paratyphosus* is similar to that of mercuric chlorid, although silver cyanids, colloidal silver, and various organic compounds which yield few silver ions on solution in water, appear to have inferior bactericidal properties. Argyrol is at least ten times less bactericidal than silver nitrate, since, in a final 5 per cent. concentration in a medium of blood serum and muscle extract, it failed to sterilize completely in six hours, whereas a final concentration of 0.3 per cent. silver nitrate kills off about 95-per cent. of staphylococci and other organisms in this period. Smith[10] tested the effect of a double salt of methylene-blue and silver nitrate on inhibition of growth of human tubercle bacilli on broth cultures and also as a chemotherapeutic agent in experimental tuberculosis in guinea-pigs, and observed complete inhibition in dilution of 1:100,000; but its chemotherapeutic action was negligible. DeWitt and Sherman[11] have also investigated the tuberculocidal properties of silver nitrate and combinations with trypan-blue, but without observing noteworthy effects.

Selective Bacteriostasis of Compounds of Copper, Gold, Tin, Zinc, Arsenic and Iodin.—Insofar as *compounds* of *copper and gold* are concerned, most attention has been devoted to their effects upon tubercle bacilli and it may be that they possess in slight degree some selective effects for these organisms. But compounds of copper and gold are as a whole relatively feeble bactericides and especially in comparison with the compounds of mercury. Copper compounds do not display any particular selective bacteriostatic properties unless it be for typhoid and Gram-negative bacilli in water in the absence of excessive amounts of organic matter, being about one-half as bactericidal as mercuric chlorid; copper sulphate, however, does possess a remarkable affinity for many species of algae, being capable of destroying them in water in 1:1,000,000 dilution.

Compounds of *gold* have always held a place of interest in relation to the chemotherapy of tuberculosis since Koch,[12] in 1890, observed that the cyanid completely inhibited the growth of tubercle bacilli in dilutions of 1:2,000,000, although it was entirely without curative effects in experimental tuberculosis of guinea-pigs. Since then a large number of new gold compounds have been tried in the treatment of tuberculosis of the lower animals and human beings, but here it may be stated that DeWitt and Sherman found that 1:1000 gold chlorid was required to kill tubercle bacilli *in vitro* and that even 1:100 of the tricyanid was not always able to kill all of the organisms, although 1:2,000,000 of the cyanid proved bacteriostatic, as stated by Koch.

Apparently compounds of *tin* and *zinc* have not been studied from the standpoint of selective bacteriostasis, but, according to the results of my own limited studies, the ordinary inorganic salts do not display any evidences of this property except that they are slightly more effective against the Gram-positive cocci like staphylococci, streptococci and pneumococci than against Gram-negative bacilli like *B. coli communis* and *B. typhosus*.

Various organic compounds of *arsenic* have long been used in the treatment of bacterial infections, but more for their possible dynamogenic than bactericidal effects. As previously mentioned, arsphenamin and neoarsphenamin display feeble bactericidal activities *in vitro* in water or saline solutions and possibly these compounds are somewhat more bactericidal for streptococci than staphylococci, but the differences are only slight and easily accounted for by other factors. The investigations of Douglas and Colebrook and Allison show that these compounds are bactericidal *in vitro* and apparently may raise the bactericidal activity of the blood for a few hours after the intravenous injection of large doses; this is particularly true of neoarsphenamin, but apparently less marked with arsphenamin and almost absent with sodium cacodylate, although the latter compound has been reported by Bargen as distinctly curative in experimental streptococcus infections of rabbits.

Iodin and compounds of iodin are worthy of brief mention in relation to tuberculosis, since they have been long advocated by some physicians in the treatment of this disease. Cantani[13] has stated that iodin in concentration of 1:500 to 1:1000 exerted some bacteriostatic effect upon tubercle bacilli, but DeWitt and Sherman found that Lugol's solution had very little bactericidal activity, since in full strength the solution was unable to kill all bacilli. Tincture of iodin has a well marked bactericidal effect upon staphylococci and streptococci, accounting for its popularity as a disinfectant of the skin and for the treatment of bacterial infections by local application, but its effects *in vivo* are very much reduced; to the best of my knowledge it does not display any particular selective bacteriostasis and certainly not for *Sp. pallida* or *B. tuberculosis*.

Selective Bacteriostasis of Creosote, Guaiacol and Their Derivatives.—It is necessary to refer briefly to the bacteriostatic and bactericidal activity of these compounds in relation to the chemotherapy of tuberculosis of the lungs, since they have long held a place of interest in the treatment of not only this disease but also in the treatment of non-tuberculous bronchitis; furthermore the question of their possible specific bactericidal effects for tubercle bacilli, as well as for the organisms of simple bronchitis and secondary infection of tuberculous tissues, such as streptococci, staphylococci, pneumococci, etc., becomes one of importance in relation to chemotherapy. I may state that Ehrlich was impressed with the possibilities of improving creosote therapy and with the assistance of Bechhold prepared a series of new compounds and added very materially to our knowledge of the chemistry of these substances in relation to their bactericidal activity, to which I will later refer. DeWitt, Suyenaga and Wells[14] have exhaustively reviewed the literature on this subject of bactericidal activity of these compounds *in vitro* and *in vivo* and have likewise contributed a thorough study of the subject themselves, so that it may suffice to quote here from their conclusions. They state that "despite the extensive use of creosote and related compounds in the treatment of tuberculosis, practically no evidence exists as to the susceptibility of *B. tuberculosis* to antiseptics of this class, or as to their influence on the course of tuberculosis in experimental animals."

Selective Bacteriostasis of Chaulmoogra Oil and Derivatives.—Interest in the possible selective bacteriostasis of *chaulmoogra oil* and its derivatives for acid-fast bacilli, and particularly *B. tuberculosis*, has been stimulated by the reports of Heiser and others concerning the curative properties of these substances in leprosy. In 1915, Hernandez[15] reported that the addition of 2-per cent. chaulmoogra oil to culture mediums inhibited the growth of tubercle bacilli and had a favorable influence on tuberculosis in a few patients. Walker and Sweeny[16] have reported that the sodium salts of the total fatty acids of chaulmoogra oil (chaulmoogrates) possess an extremely high bactericidal and bacteriostatic activity for the tubercle bacillus *in vitro*. The authors also give a complete review of the literature and a description of the chemistry of chaulmoogra oil. At about the same time Davis, Jager and I[17] were experimenting with oil expressed cold from seeds of *Taraktogenous kurzii* and bovine tubercle virulent from guinea pigs. Since the oil was immiscible with the medium, a special technic was required for determining its possible germicidal activity for the tubercle bacillus.

Our experiments were entirely negative; even pure undiluted chaulmoogra oil failed to kill all of the tubercle bacilli and the results are in striking contrast to those of Walker and Sweeney, but our work was conducted with the whole oil, while they employed the soluble sodium salts of the total fatty acids. Out next experiments were made with what may be called a combined *in vivo-in-vitro* technic, aiming to bring the oil into more intimate contact with the bacilli and testing for germicidal activity by injecting the mixtures into guinea-pigs. This method was rather favorable to any germicidal activity of the oil, as it was brought into close contact with the bacilli for eighteen hours at room temperature. The number of bacilli was not large and the dose injected into the pigs required 5 to 6 weeks to produce well marked inguinal adenitis and infection of the spleen and abdominal glands. However, even if but few bacilli escaped destruction, the results of inoculation into the guinea-pigs would probably produce tuberculosis; therefore, these experiments have shown the absence of complete bactericidal activity, but have yielded no information on the question of partial destruction of the bacilli. While I shall later refer to the results of additional experiments

which we conducted on the treatment of experimental tuberculosis of guinea-pigs with chaulmoogra oil, I may state here that our results were completely negative.

Bactericidal Activity of Hexamethylenetetramin and the Selective Bacteriostasis of Its Quaternary Salts.—Since the recognition in 1894 of *hexamethylamin* as a beneficial substance in the treatment of bladder infections, it has been recommended as an antiseptic agent for virtually all the body fluids, largely on the basis of the fact that after internal administration the drug may be found present everywhere in the body. To this circumstance is added the advantageous feature that hexamethylenamin is practically non-toxic. It is an established fact, however, that this substance as such is not bactericidal, since Hanzlik and Collins[18] clearly demonstrated that bacterial growth is not prevented by it, even in proportions much higher than could obtain anywhere in the body, and that the administration of hexamethylenamin could not be expected to exert a marked antiseptic effect in the tissues, although a partial restraining action was not excluded. In an acid solution, hexamethylenamin disintegrates into ammonia and formaldehyd. The latter substance is admittedly bactericidal and to it doubtless are due the beneficial therapeutic results experienced in the treatment of urinary infections; for the urine usually develops an acidity favorable to the desired reaction, liberating formaldehyd. The latest effort, by De Eds,[19] to discover an unsuspected comparable change in the migration of hexamethylenamin through the blood and tissues has yielded only negative results. Jacobs, Heidelberger, Amoss and Bull,[20] however, have thought it worth while to synthesize new compounds of hexamethylamin and they have studied these with particular care for evidences of selective bacteriostasis for *B. typhosus*, streptococcus, meningococcus, and gonococcus with some striking instances of partial specificity. A particularly valuable outcome of their work has been to secure new information bearing upon the important question of chemical constitution of the quaternary salts of hexamethylamin to disinfectant activity *in vitro*.

Bactericidal Activity of the Blood and Urine after the Administration of Mercurial and Arsenical Compounds.—The effect of compounds of mercury and other metals in the treatment of experimentally produced bacterial infections may be now considered, but before taking up a consideration of this important subject, it will be profitable to inquire whether the administration of these substances to the lower animals appreciably raises the bactericidal activity of the blood; furthermore, the matter of rendering the urine bactericidal is also of interest and importance and especially in reference to the treatment of infections of the urinary system.

Schamberg, Raiziss and I[21] have observed that the intravenous injection of mercurophen into rabbits in sublethal amounts (0.0015 gm. per kilo) was found to increase appreciably the bactericidal activity of the blood during the first few hours for staphylococci, pneumococci and *B. typhosus*, whereas the administration of mercuric chlorid and other mercurial compounds usually failed to increase to the same degree the bactericidal activity of the whole blood of rabbits for these microorganisms. Similar results were observed by Borow and myself[22] employing the Heist-Lacy method.

Hill and Colston[23] have observed that, after the intravenous administration of mercurochrome to normal rabbits, the urine became either bactericidal or strongly bacteriostatic for *B. coli*. This inhibitive action was strongest during the first five hours after injection. The same authors[24] have shown that, after the intravenous administration of mercurochrome to normal

rabbits, the blood is strongly bacteriostatic for *B. coli* from fifteen to forty-five minutes after injection.

The results of these investigations have indicated, therefore, that it is possible to administer sufficient amounts of some of the new mercurial compounds, and especially mercurochrome, to appreciably raise the bactericidal effects of the blood. It is true that these effects are of short duration because of rapid elimination and fixation of the compounds by the tissues, but yet it is sufficient to lend considerable basis for the hope that it may be possible to appreciably reduce the degree of a bacteremia or dissipate it entirely by the intravenous injection of large but comparatively safe doses of these compounds; in fact, the results of animal experiments have shown that this may occur in at least some instances.

I have already referred to the bactericidal activity of arsphenamin and neoarsphenamin *in vitro* in water or saline solution. Douglas and Colebrook[25] state that the bactericidal activity is reduced in the presence of serum or blood. They also reported that the intravenous injection of neoarphenamin in ordinary doses raises the bactericidal activity of the blood which reaches a maximum in one hour and rapidly diminishing in three or four hours. Curiously arsphenamin in ordinary dosage was reported as being without these effects, but the authors suggest that neoarsphenamin may be of value in the treatment of bacterial infections, an opinion which later induced Allison to employ these compounds in experimental streptococcus bacteremias of rabbits.

Compounds of Mercury, Arsenic, Gold, Copper, Iodin, and Other Substances in the Chemotherapy of Experimental Tuberculosis.—With this review of the bactericidal effects of compounds of mercury, arsenic and other metals *in vitro* and in relation to the bactericidal properties of the blood *in vivo*, their influence upon experimental bacterial infections of the lower animals may now be considered. I may state at once that the results have been largely disappointing and of a negative character, but yet within the past few years some compounds belonging to this class have yielded encouraging results.

Since tuberculosis was one of the first diseases to be produced experimentally and since the disease is so readily induced in guinea-pigs, it is no wonder that the major portion of experimental work in chemotherapy with mercury and other metals was conducted with this infection since Koch's and Cornet's original experiments in 1890, the former reporting that mercury in vapor form was entirely without influence on the progress of the disease in animals and the latter observing that mercuric chlorid, administered to guinea-pigs to the point of toxic effects followed by inoculation with living bacilli, had no appreciable curative influence when compared with the induced disease in control animals which were not mercurialized.[26]

Mercurial Compounds in the Chemotherapy of Experimental Tuberculosis.—Insofar as the ordinary and well-known organic and inorganic *salts of mercury* are concerned, it may be stated that none have shown any appreciable curative effects upon tuberculosis produced in guinea-pigs. In 1914, DeWitt[27] found that an insoluble mercury-trypan-blue had some influence upon the disease, since the treated animals lived longer than the controls although the compound was quite toxic. In a subsequent study DeWitt[28] employed a large number of new compounds in the treatment of experimental tuberculosis of guinea-pigs, with practically negative results. Later DeWitt[29] used mercurophen and mercurochrome in the treatment of guinea-pigs inoculated with tuberculosis, with the general result that these compounds seemed to delay considerably the development of the disease; but marked generalized

tuberculosis occurred in all the animals that lived long enough. Of interest in this connection are the results of Corper's experiments with dogs indicating that mercurochrome in one-half to two per cent. solution should not be injected into tuberculosis cavities or into the pleural spaces in the treatment of tuberculosis or other infections because of the extensive tissue changes produced, largely in the nature of hemorrhagic pneumonitis with focal abscesses and terminating in proliferative pneumonitis.

Arsenical Compounds in the Chemotherapy of Experimental Tuberculosis.—Although *arsenic* has long been advocated as beneficial in the treatment of tuberculosis, Arken and Corper[30] concluded in 1916, after a review of the literature, that it had not been demonstrated to have any specific action and that its value in this disease can be attributed only to its favorable effects on metabolism. These investigators also studied the effects of sodium arsenite, sodium cacodylate, mercury cacodylate, atoxyl, arsacetin and neosalvarsan in the treatment of inoculated animals, with negative results.

Gold Compounds in the Chemotherapy of Experimental Tuberculosis.—Probably no compounds of the heavy metals have commanded as much attention in the treatment of tuberculosis as the *salts of gold;* indeed, quite a large literature has accumulated upon the subject insofar as the treatment of tuberuclosis of human beings is concerned, which is reviewed in Chapter VIII, but here it may be stated that relatively few experiments have been conducted with experimental tuberculosis of the lower animals and the results of these have been largely of a negative character. Koch, in 1890, observed that the gold salts then available had no beneficial effects in tuberculous animals, but interest in these compounds was greatly renewed by Spiess and Feldt[31] who, together or separately, between 1912 and 1916, published a series of papers on the subject. These authors found that gold cyanid and gold potassium cyanid were too toxic for use in human patients, but, as cantharidin had earlier been found to have a marked affinity for tuberculosis tissues, they conceived the idea of reducing the toxicity of the cantharidin and using it as a carrier for the specific gold cyanid; apparently they were able to reduce the toxicity of the cantharidin without altering its affinity for tubercles by condensing it with ethylenediamin. This compound was quite nontoxic and combined readily with gold cyanid. They used first the auric salts, but finding them less efficacious than the aurous salt, employed mono-canthatidyl-ethylene-diamin-aurous-cyanid under the name "aurocantan" or "canthoridyl."

This compound was first tested on about 100 animals during 1½ years and was then used on human patients. They claim that its beneficial effects are due partly to a direct inhibitory action on the tubercle bacilli and partly to an indirect action on the defensive agencies of the body believing that it simulates connective tissue formation and destroys disease products by its oxidizing power. In experimental tuberculosis in animals, they claim that weight was increased and life was prolonged.

But a number of other investigators found none or much less beneficial effects than those reported, and Pekanovitch,[32] Hauck,[33] Gelpe[34] and others have directed attention to the dangers attending the use of "aurocantan" from the destruction of erythrocytes and hemorrhages with the production of jaundice and anemia. Furthermore, DeWitt, Cadwell and Leavell,[35] in a series of studies employing "aurocantan" and the simple gold cyanids, observed that gold was deposited in the various organs of experimental animals including tuberculous tissue, but without appreciable curative effects. Indeed, the amounts found in the liver, spleen, lungs and other sites favored by tubercle formation were many times the concentration necessary in the

test-tube completely to inhibit growth of the bacillus, but yet excited no restraining influence upon the disease.

At this writing a new gold compound designated as "sanocrysin" and produced by Møllgaard in Copenhagen is attracting considerable attention in the treatment of tuberculosis. Møllgaard states that no reaction follows the introduction into the body of this remedy by the intravenous method, provided the tissues are healthy; but if they are tuberculous, a violent reaction ensues. It is assumed that the reaction is the result of release of toxic substances from tubercle bacilli that are destroyed by the remedy. The reaction is opposed by the injection of a specially prepared horse serum which, Møllgaard believes, has greatly reduced the severity of the phenomena of the reaction. Møllgaard has described the complete clinical recovery of two monkeys with severe tuberculous infection, following the use of his method and several well-known Danish clinicians have reported brilliant results in the cases of many patients. However, there were several instances in which death had followed the administration of the remedy, presumable on account of difficulty in determining accurately the proper dosage, and a final opinion of its value cannot now be expressed. I may state however, that at the present writing the results of carefully conducted experiments in the Hygienic Laboratory by a commission composed of Theobald Smith, W. H. Park and E. C. Schroeder have failed to demonstrate any marked degrees of curative activity by this compound. From our knowledge of the action of gold compounds in general in tuberculosis, I rather surmise that the toxic reaction produced by "sanocrysin" is due to foci poisons from the lesions engendered by the tuberculin-like reaction of hyperemia, exudation and enhanced cellular necrosis; possibly any curative effects it may possess are likewise similar to those produced by these tuberculin-like reactions favoring the laying down of fibrous tissue and without involving the question of direct and marked tuberculocidal properties of the compound itself.

Copper Compounds in the Chemotherapy of Experimental Tuberculosis.—*Copper* is another metal which has attracted considerable attention in the treatment of tuberculosis, especially of human beings, but without much support from the standpoint of their effects in experimental infections. DeWitt[36] found that copper trypan-blue did not penetrate either normal or tuberculous tissues, and that it is probably changed to an insoluble form or a suspension colloid and retained at the point of injection. Von Linden,[37] however, believes that copper is bound by the fatty acids of tubercle bacilli and that tuberculosis of guinea-pigs treated with copper shows a more chronic course than untreated controls, with the complete checking of the disease when large initial doses (3 to 5 mgm. of copper) are given. Other investigators, however, have not been able to confirm these results, among them being Selter,[38] who was associated with V. Linden, and who states that there was none or but very little favorable influence of copper on tuberculosis and none that could be called specific for the disease. Corper,[39] who has reviewed this literature up to 1912, made a very careful study of the absorption, distribution and curative activity of copper compounds in tuberculous guinea-pigs and rabbits, finding that they are distributed not as salts but in colloidal form and that they do not usually penetrate tubercles or appreciably influence the disease. Koga,[40] however, working with a combination of two parts of potassium cyanid and one part of copper cyanate, the mixture being designated as "cyanocuprol," reported good results in the treatment of tuberculosis of guinea-pigs. This compound has been extensively used in Japan and with apparently good results in some cases, but it is now the consensus of opinion that the effects are not specific but due to the focal

tuberculin-like reactions which may be produced; in my experiments employing infected guinea-pigs treated with cyanocuprol sent to me by Koga, the results were almost negative, only an occasional animal surviving long enough after the death of the controls or showing a sufficiently milder course of the disease, to justify the conclusion that some slight beneficial effects may have been produced. No doubt copper compounds may favorably influence tuberculosis, but this appears to be non-specific and the result of some radicle of the compound other than copper itself producing hyperemia and inflammation exciting effects about the tubercles similar to the effects of tuberculin.

Iodin in the Chemotherapy of Experimental Tuberculosis.—Iodin and iodin preparations have also long held a place of interest in the treatment of tuberculosis. But iodin has but a feeble bactericidal action on tubercle bacilli and little is to be expected from the standpoint of specific tuberculocidal effects *in vivo*. However, it is possible that iodin may serve as a carrier of more potent chemical antiseptics into tubercles and in truth the substance has commanded most attention from this aspect in the modern chemotherapeutic investigations in tuberculosis. In 1907, Loeb[41] and Loeb and Michaud[42] reported that tuberculous tissue took up a disproportionately large amount of iodin administered as potassium iodid and Wells and Hedenburg,[43] after an exhaustive study of this subject in 1912, were able to confirm these findings but showed that the process was not specific since other necrotic tissues also took up more iodin than normal tissues. The explanation offered was that normal cells are not perfectly permeable to iodids (except perhaps kidney cells) and lose this impermeability or semipermeability when killed or injured, thus becoming entirely permeable for crystalloids present in the surrounding fluids. No evidence could be found of any tendency for iodin compounds of whatever nature to accumulate in tubercles or other necrotic areas, or to persist in such areas when disappearing from the normal tissues and the blood. Exudates contained approximately the same proportion of iodin as the blood of the same animals, and hence any area with inflammatory edema and congestion will commonly show more iodin than normal tissues, although not usually more than the blood.

While several investigators have claimed that the daily administration of potassium iodid increases absorption and the organization of tuberculous nodules, these claims have not been confirmed and, indeed, Hirsch[44] and others have concluded that the use of iodin and iodids for facilitating the absorption of necrotic material and promoting the organization of tuberculous and other granulation tissue has no experimental support. As stated by DeWitt, iodin as a chemotherapeutic agent has as yet given little proof of its value or encouragement for the hope that it may prove of real value in the chemotherapy of tuberculosis.

Cerium, and Other Rare Earths in the Chemotherapy of Experimental Tuberculosis.—At the present writing the salts of *cerium, samarium, neodymium, praseodymium and other rare metals* are commanding considerable attention in the chemotherapy of tuberculosis and especially by French physicians. Grenet and Frouin,[45] for example, state that it has been established that the salts of cerium reduce the fats of the tubercle bacillus from 25 to 40 per cent. to 22 to 16 per cent. in cultures to which the salts are added, and that these salts induce an increase of lymphocytes both in men and animals, but that some are too toxic for clinical use. Renon[46] goes so far as to state that "the results obtained by Grenet and Frouin with the sulphates of the cerium earths are the most scientific yet realized in the chemotherapy of tuberculosis." Beneficial effects have been claimed in tuberculous patients and guinea-pigs which they ascribe to an effect upon the bacillus as well

as by favoring sclerosis of tubercles on account of the enormous mononuclear leukocytosis induced. They have not claimed complete cures, however, and insist that the salts must be used only in afebrile cases of pulmonary tuberculosis. Frouin[47] states that rabbits and guinea-pigs inoculated with virulent bovine tubercle bacilli and treated with intraperitoneal injections of sulphates of cerium earths, have shown sclerosis of the lesions and with few exceptions have lived from two to five months longer than the controls but DeWitt[48] has recently stated that the experimental observations on the cerium earth group do not warrant the amount of clinical enthusiasm that has been reported.

Creosote, Guaiacol, and Their Derivatives in the Chemotherapy of Experimental Tuberculosis.—The results of bacteriostatic and bactericidal tests *in vitro* for human and bovine tubercle bacilli employing *creosote, guaiacol and their derivatives*, have not given much or any hope for specific bactericidal effects *in vivo* and the results of animal tests have been generally a negative character as shown by the review of the literature by DeWitt and her colleagues[49] of these compounds as well as the "thiocols" and other derivatives. They also made therapeutic tests employing guinea-pigs injected subcutaneously with two strains of human tubercle bacilli, one highly virulent and the other much less so and failed to observe any beneficial therapeutic effect on tuberculous guinea-pigs, which, in view of the low bactericidal power of the substances test was to be expected. As stated by them it does not mean that these substances may not have value in open tuberculous infections of man in which other bacteria than *B. tuberculosis* are involved, but it does substantiate the opinion that seems to have been generally reached by careful clinical observers, that creosote and guaiacol do not have a specific action on tuberculous infection.

Chaulmoogra Oil and Its Derivatives in the Chemotherapy of Experimental Tuberculosis.—As previously stated, Davis, Jager and myself found that undiluted and diluted *chaulmoogra oil* had no appreciable bactericidal effect on virulent tubercle bacilli as determined by an *in vitro-in-vivo* method employing guinea pigs. In doses of 0.2 c.c. per 100 gm. of body weight administered by intramuscular injection at weekly intervals (equivalent to 2 c.c. per kilo or 120 c.c. per 60 kilos) it had none or but slight effect on the course of tuberculosis in infected guinea-pigs. The oil was found, however, to be relatively non-toxic for guinea-pigs; animals have borne at least 11 intramuscular injections of 0.2 c.c. per 100 gm. without deleterious effect except localized inflammatory changes at the sites of injections.

Walker and Sweeney, however, have reported that the fatty acids of the chaulmoogric series, chaulmoogric and hydnocarpic acids and possibly lower isomers of this series may have some curative effects in experimental tuberculosis of rabbits and guinea-pigs since the average duration of life of the treated animals was greater than that of the untreated controls. Culpepper and Ableson[50] also reported some beneficial effects in the treatment of tuberculous guinea-pigs with the acid sodium salts of the esters of chaulmoogra oil, but Voegtlin, Smith and Johnson[51] after exhaustive tests with chaulmoogra oil, chaulmoogric acid, and ethyl esters and iodin saturated ethylchaulmoograte in guinea-pigs inoculated with a virulent strain of human tubercle bacilli, reached the conclusion that these substances had no influence upon experimental tuberculosis. Finally, Walker has reported that he was unable to find chaulmoogra oil and its fatty acids in the tubercles of treated animals and he considered this failure to penetrate tuberculous tissues the cause of the negative results in the treatment of the disease with these substances.

Cinnamic Acid and the Cinnamates, Bile and the Bile Salts, Fats and Fatty Acids and Various Hydrocarbons in the Chemotherapy of Experimental Tuberculosis.—Finally, brief mention may be made of *cinnamic acid* and *sodium cinnamate* in the treatment of experimental tuberculosis since, as early as 1893, Landerer[52] claimed that the former caused a marked increase of leukocytes around tubercles in animals; Hacobson[53] states that the same effects were produced by *ethyl cinnamate* along with a shortening of the thermal reaction time following injections of tuberculin. Corper[54] however, found that *sodium cinnamate* was too hemolytic and toxic for the treatment of tuberculosis.

In 1909, Deycke and Much[55] reported that tubercle bacilli suspended in 25 per cent., solutions of *cholin* underwent partial bacteriolysis with a complete solution of the fatty material with loss of the Gram staining power; on this basis, Mehler and Ascher[56] tried "*borcholin*," a cleavage product of cholin, and reported that it possessed tuberculocidal activity *in vitro* and was well borne by animals by intravenous injection, but with little or no effect in the treatment of tuberculosis. It was hoped, however, that "borcholin" might serve as a carrier of more active agents and success was claimed in the healing of tuberculous lesions by combining it with copper; Mayer[57] states that a combination with gold potassium cyanid apparently aided the curative properties of the latter.

More recently Takeoka,[58] drawing attention to numerous reports in literature indicating that the liver may be actively concerned in the destruction of tubercle bacilli and other organisms *in vivo*, tried the effects of *taurin*, the most characteristic animo-acid of the bile, in dose of 0.05 to 0.1 gm. in the treatment of experimental tuberculosis of guinea-pigs and rabbits. Taurin was also used in combination with a colloidal mixture of sodium gold chlorid and sodium oleate, but equally good results were observed with the taurin alone. It was claimed that in nearly every instance the untreated controls died before any of the treated animals and that in some instances apparent cures were brought about. This work, however, has not yet been confirmed and verification is required before one may accept the conclusion that bile or bile salts have a marked therapeutic value in experimental tuberculosis, and especially since the liver may be extensively involved in tuberculous infection, indicating that the bile frequently fails to inhibit the disease in both human beings and the lower animals.

This naturally brings up the question of the curative activity of the *fats and fatty acids* in tuberculosis, and especially cod liver oil, which deservedly occupies a useful place in the treatment of the disease and especially of the surgical forms. Miller[59] states that sperm oil added to media on which tubercle bacilli were being grown caused rapid bacteriolytic changes and DeWitt has confirmed these observations. The salts of the fatty acids, however, are lacking in beneficial effects in tuberculosis suggesting that the whole oil may owe its curative properties to vitamins which bring about an accelerated rate of re-calcification and an increase of inorganic phosphorous in the blood in both rickets and tuberculosis in much the same manner as heliotherapy, without involving the question of specific tuberculocidal action from the oil which is doubtless a very remote and improbable effect.

Compounds of Arsenic, Mercury and Other Substances in the Chemotherapy of Experimental Streptococcus, Pneumococcus, Staphylococcus, Anthrax and Other Infections.—Much less investigation has been devoted to the subject of the chemotherapy of experimental pneumococcus, streptococcus, staphylococcus and other bacterial infections with compounds of the metals, although this is a very important field worthy of intensive study

because of the frequency and highly fatal character of these diseases. I have reason to believe, however, that much more work has been done than is to be found in the literature because of the hesitation in recording negative results. This has certainly been true of Schamberg, Raiziss and I who have worked with the mercurials and arsenicals in experimental bacterial infections off and on for more than six years but delaying in reporting our work because of the negative and disappointing character of the results.

I am inclined to believe that much of this has been due to the unnecessary severity of our experimental infections; for example, streptococcus and pneumococcus infections induced in mice and rabbits have usually killed in 48 hours and it would require a very powerful agent indeed to prolong life or cure the infection under such conditions. I mention these circumstances at present only to re-emphasize what was stated in Chapter III, that compounds failing to materially influence such acute virulent experimental infections may yet prove beneficial and useful in the treatment of the less severe and fulminating infections of human beings.

While a large number of compounds have been tried out in the treatment of experimental tuberculosis, as just reviewed in the preceding section, relatively few have been employed in the various infections now under discussion. Indeed, only the new organic arsenicals like arsphenamin and neoarsphenamin, some of the new mercurials like mercurophen and mercurochrome, and the hexamethylamin and a few other compounds need to be considered.

Arsphenamin and Other Arsenicals in the Chemotherapy of Experimental Systemic Streptococcus Infections.—In 1918, Allison,[60] influenced by the report of Douglas and Colebrook on the bactericidal activity of arsephenamin and neoarsphenamin *in vitro*, as well as from the standpoint of temporarily raising the bactericidal activity of the blood following intravenous injections of ordinary doses, undertook a study of the bactericidal effects of *arsphenamin in vitro* and in the treatment of streptococcus infections of rabbits. Allison employed two different strains of streptococci and found that he was able to save about 70 per cent. of infected rabbits with intravenous injections of three different brands of arsphenamin as against a survival of about 40 per cent. among untreated controls. In more acute virulent infections, however, the componds were without effect, but Allison expressed the belief that the treatment may be successful in human infections if the source of infection could be removed or drained and the injections given before secondary localization occured.

During the past four years I have conducted a large number of tests with the assistance of Dr. Malcolm J. Harkins. Rabbits and mice were employed, the former being infected by intravenous injections of streptococci and the latter by subcutaneous injections according to Morgenroth's method. Several different strains of streptococci were used, varying greatly in virulence for these animals. *Arsphenamin* and *neoarsphenamin* were administered by intravenous injection to rabbits and by intraperitoneal injection to mice, the doses being 0.010 gm. of the former and 0.015 gm. of the latter, per kilo of weight. The first dose of each drug was given four hours after inoculation and subsequent doses at daily intervals. Blood cultures were made of the rabbits during life and of all animals after death; numerous untreated controls were always included. The results have varied according to the virulence and severity of the infection. In acute fulminating infections with positive blood cultures ending fatally in four days or less, we have never been successful in sterilizing the blood or appreciably prolonging life. In less virulent infections and especially among mice, both arsphenamin and

neoarsphenamin have prolonged the lives of a small percentage of animals, although in no case in which streptococci were found in the blood stream. I believe, therefore, that arsphenamin and neoarsphenamin may exert an appreciable but relatively feeble curative effect upon experimental streptococcus infections, but that these effects are not appreciable in the presence of infections sufficiently virulent to maintain the organisms in the blood.

I have also experimented with *sodium cacodylate* in the same manner, because of the reports bearing upon its use in the treatment of streptococcus endocarditis, but in my experience the compound has proved without appreciable effect in doses as high as 0.005 gm. per kilo (corresponding to 5 grains per 60 kilos) by administration according to the above plan.

Arsphenamin and Neoarsphenamin in the Chemotherapy of Experimental Anthrax.—Probably no bacterial infection has attracted as much attention as *anthrax from the standpoint of treatment with arsphenamin and neoarsphenamin;* quite a large literature has accumulated upon the subject most of it dealing with the results observed in the treatment of infections of human beings. Schuster,[61] observed in 1912 that rabbits inoculated subcutaneously with lethal amounts of anthrax culture could be saved if arsphenamin in dose of 0.04 gm. per kilo was given within the following 12 hours; smaller doses were generally ineffective. Bierbaum[62] reported that, if arsphenamin in dose of 0.1 gm. per kilo by subcutaneous injection was given to guinea-pigs immediately after inoculation with anthrax bacilli, the majority survived; but if treatment was delayed for two to four hours, the majority perished of anthrax bacteremia. Better results were observed with a combination of arsphenamin and anti-anthrax serum. Isabolinsky[63] has also reported that arsphenamin in dose of 0.06 gm. per kilo apparently protected rabbits against lethal anthrax injection when the treatment was given not later than two hours after inoculation. When the drug was combined with anti-anthrax serum the results were more marked, since animals could be saved up to seven hours after injection.

During the past six years, I frequently experimented with arsphenamin and neoarsphenamin in the treatment of mice, guinea-pigs and rabbits inoculated with virulent anthrax bacilli, but with generally unsatisfactory results largely due to the difficulty of gauging the correct amount of culture to employ. The anthrax bacillus in my experience has proved one of the most difficult to manage from this standpoint, since it is subject to rather wide and unexpected changes in virulence for the lower animals. In my experiments, arsphenamin and neoarsphenamin were administered four and two hours before inoculation, simultaneously with inoculation and two, four and twenty-four hours after inoculation. In a general manner, it may be stated that arsphenamin in dose of 0.02 gm. per kilo and neoarsphenamin in dose of 0.03 gm. per kilo have saved some animals when given two hours before or within two hours after inoculation with approximately ten times the minimal lethal dose of culture. Smaller doses of the drug were generally without effect; larger doses actually gave poorer results probably because of a lowering or resistance by the toxic effects of the drugs. Treatment four hours before inoculation sometimes saved the animals; in most instances, however, it failed; treatment four hours or longer after inoculation usually failed to prolong life. I believe it may be stated, therefore, that arsphenamin and neoarsphenamin, especially the former, possess in slight degree an appreciable bactericidal activity for the anthrax bacillus *in vivo,* but the curative effects are so feeble that it is doubtful if these compounds are capable of favorably influencing anthrax bacteremias in man.

Mercurial and Arsenical Compounds in the Chemotherapy of Experimental Systemic Pneumococcus Infections.—Owing to the high bacteriostatic and bactericidal activity of *mercurophen* for pneumococci *in vitro*, Schamberg, Raiziss and I[64] tried out its curative activity *in vivo* employing since inoculated with amounts of virulent Type I pneumococci lethal within four days; the results, however, were negative, since we were unable to appreciably prolong the lives of these animals or check the inevitable bacteremias.

A year previous Steinfield and I[65] had tried a large number of new mercurials including mercurophen but with negative results. I have also employed *mercurochrome* in dose of 0.005 mg. per kilo (approximately one-eighth of the maximal tolerated dose) in similar experiments, but with negative results although occasionally the lives of mice treated simultaneously with inoculation or within two hours thereafter were prolonged from one to three days beyond the controls. I have also used *arsphenamin* in dose of 0.02 gm. per kilo and *neoarsphenamin* in dose of 0.03 gm. per kilo, in similar tests with negative results.

It is to be admitted, however, that these experiments were severe tests for the curative activity of the compounds, although fashioned after the method for determining the curative activity of antipneumococcus serum. Recently Dr. Malcolm J. Harkins and I did over again some of the work, employing rabbits infected with repeated minute amounts of culture of virulent pneumococci lethal in about eight days. Treatment with mercurophen (0.005 gm. per kilo) and arsphenamin (0.03 gm. per kilo) by intravenous injection was without effect, the first dose of each drug being given four hours after inoculation followed by daily injections; mercurochrome occasionally prolonged the lives of some animals a few days, but all succumbed with pneumococcus bacteremia. I have not employed these compounds in the treatment of experimental pneumococcus pneumonia of rabbits or monkeys, but on the basis of these results I doubt if the arsenicals and mercurials employed in our tests would exert appreciable degrees of curative activity.

Mercurial Compounds in the Chemotherapy of Experimental Streptococcus Systemic Infections.—In 1920 and at various times since then, I have experimented extensively with mercurophen and other new organic compounds of mercury in the treatment of acute infections of rabbits produced with virulent strains of hemolytic streptococci, but with uniformly negative results. As a general rule, the untreated animals succumbed within five days after inoculation and almost unvariably with a severe bacteremia; the treated animals and especially those receiving intravenous injections of the compounds within two hours of the time of infection, sometimes lived a day or two longer, but none were saved although I am confident that the blood stream was frequently sterilized for brief periods following the injections of compounds.

In 1922, however, Piper,[66] reported experiments with rabbits infected with streptococci and treated with intravenous injections of mercurochrome in dose of 0.005 gm. per kilo, indicating that this compound had an appreciable bactericidal activity in the blood and in one instance, at least, apparently cured a severe local infection of an eye. On the basis of this word Piper began the treatment of puerperal sepsis with mercurochrome and with encouraging success, which I shall discuss in Chapter VIII.

Dr. Harkins and I have repeated Piper's experiments but not with very satisfactory results. I realize, however, that the strain of streptococcus employed by us may have been too highly virulent, since rabbits succumbed in thirty-six hours with extremely severe bacteremias. In some experiments

the dose of culture was so reduced that the animals lived for longer periods, but in some of these the untreated controls lived indefinitely and thereby rendered the result difficult of proper interpretation. But we did observe some effect upon bacteremias, although the sum total of experiments employing dozens of infected rabbits were disappointing insofar as complete cures were concerned.

We then turned our attention to experiments employing white rats, since the higher natural immunity of these animals for streptococci prevented the occurrence of overwhelmingly severe infections, but here again the results were largely of a negative character, although occasionally hopeful results were observed when the compound in dose of 0.005 gm. per kilo was given within an hour or two after inoculation and followed up by daily doses.

Better results, however, were observed with mice infected by subcutaneous injections of streptococci according to Morgenroth's method described in Chapter IV. As a general rule, we inoculated the mice every three days and produced thereby a streptococcus cellulitis with infection of the blood stream lasting from seven to ten days and more closely duplicating conditions as encountered among human subjects with streptococcus infections. Mercurochrome was given by intraperitoneal injection in dose of 0.005 gm. per kilo, at daily intervals and four hours after each inoculation. Controls receiving the drug alone lived indefinitely; untreated controls succumbed with severe streptococcus cellulitis and bacteremias, whereas 30 per cent., and sometimes more of the treated animals lived indefinitely and showed no local lesions at the site of inoculation. In my experience, this technic has proved the most satisfactory of all employed and I believe that mercurochrome demonstrated unmistakably some degree of curative activity. Curiously enough larger doses of mercurochrome up to 0.010 gm. per kilo, did not improve the results and indeed, animals treated with this amount sometimes died earlier than the untreated controls, which we ascribed to the toxic effects of the compound acting primarily as a depressor of resistance.

Salicylates and Related Substances in the Treatment of Experimental Streptococcus Arthritis of Rabbits; the Toxicity of the Salicylates.—One of the unsolved mysteries of therapeutics is the mechanism of the beneficial and even curative effects of the salicylates and related substances in acute rheumatic fever. Indeed, these effects are frequently so striking that many physicians have included the salicylates in this disease in the category of the specific chemotherapeutic remedies and I am, for this reason, including here a description of recent experiments bearing upon the antibacterial properties of these compounds *in vivo* for streptococci.

The etiology of acute rheumatic fever, however, has not yet been solved although the majority of investigators in this field regard the disease as a bacterial infection and probably due to some member of the streptococcus group (*Diplococcus rheumaticus*). The relation of this organism to the etiology of rheumatic fever has been thoroughly reviewed by Swift and Kinsella[67] and need not be here discussed; Miller[68] has also recently reviewed the subject and reported an extensive series of attempts to transmit the disease to rabbits and guinea-pigs by inoculation with whole blood, serum, joint fluid, pleural fluid, throat washings, and suspensions of tonsil tissue of patients, with negative results insofar as the joints were concerned, although myocardial lesions were apparently produced in one-half to two thirds of the animals. It must be admitted, therefore, that the etiology of acute rheumatic fever is as yet undetermined but on the other hand there can be no reasonable doubt of the occurrence of more chronic forms of arthritis due to streptococci and presumably as focal infections.

Therefore, the experiments reported by Davis,[69] Fantus, Simmonds, and Moore,[70] Swift and Boots[71] in the production of arthritis in rabbits with hemolytic and non-hemolytic streptococci and its treatment with salicyl compounds are of interest. After an extensive series of experiments they concluded that rabbits inoculated intravenously with non-hemolytic streptococci, while under the influence of full therapeutic doses of sodium salicylate, developed almost as many inflamed joints as the untreated controls similarly inoculated. The salicylated rabbits, on the other hand, had a much higher proportion of mildly inflamed joints than did the controls. This anti-inflammatory action was most evident in the animals inoculated with streptococci of the lowest virulence, and could not be demonstrated in animals inoculated with hemolytic streptococci.

Hanzlik and Karsner[72] have shown that sodium salicylate in doses of from 0.3 to 0.5 gm. per kilo of body weight in dogs and cats leads to severe cloudy swelling of the kidneys, and often to acute tubular nephritis. When we consider that from 0.15 to 0.2 gm. of salicyl per kilo of body weight is the maximum amount that can be tolerated for two or three days by most patients without developing severe albuminuria, it is probable that these animals used by Fantus and his co-workers had severe renal changes. On the other hand, sodium salicylate is of rather low toxicity for rats and rabbits, as I have observed these animals to withstand amounts as high as 0.3 to 0.4 gm. per kilo by single intravenous injection without apparent harm.

Mercurial and Arsenical Compounds in the Chemotherapy of Experimental Staphylococcus Infections.—As stated in Chapter IV, the only satisfactory method so far developed in my experience for producing generalized staphylococcus infections for chemotherapeutic experiments in the intravenous injection of rabbits or rats with sufficient amounts of culture of *Staphylococcus pyogenes aureus* to produce infection of the kidneys, heart, diaphragm and other internal organs and comparing the extent and degree of infection among treated animals with untreated controls. Numerous experiments of this kind employing mercurophen and other organic mercurials, arsphenamin and neoarsphenamin, have almost invariably produced negative results. Mercurochrome, however, in dose of 0.005 gm. per kilo has apparently cleared the blood stream of staphylococci in some animals and likewise sharply reduced the extent of abscess production in the internal organs, but the lesions are such that the results cannot be expressed in terms of percentage of survivals since the majority of animals do not succumb in a given period, as in experimental streptococcus and pneumococcus infections. I have had no evidence so far of mercurochrome, given by intravenous injection, sterilizing staphylococcus abscesses of the internal organs or of the skin following subcutaneous inoculation, but the drug has unmistakably sterilized the blood of experimental animals at least for brief periods of time and thereby prolonged the lives of some animals with less rapid loss of weight and other toxic effects.

Mercurial and Arsenical Compounds in the Chemotherapy of Experimental Systemic B. Coli Infections.—Since Young and his associates have found mercurochrome efficacious in the treatment of *B. coli* infections of human beings, Dr. Harkins and I have endeavored to study its effects in experimental *B. coli* infections of rabbits. A culture was employed, which upon intravenous injection, proved fatal for rabbits in approximately five days with bacteremia (positive blood cultures). Treatment with mercurochrome by intravenous injection in dose of 0.005 gm. per kilo, the first dose being given four hours after inoculation followed by several daily doses, prolonged the lives of some animals for eight days and in a few instances for indefinite periods.

We have not conducted a sufficient number of these tests, however, to warrant definite statements but believe that enough has been done to show that mercurochrome may favorably influence rather severe experimental *B. coli* infections. Similar experiments employing mercurophen in dose of 0.001 gm. per kilo and arsphenamin in dose of 0.01 gm. per kilo, yielded negative results.

Arsphenamin and Neoarsphenamin in the Chemotherapy of Experimental Swine Erysipelas Infections.—In 1917, Bierbaum[73] reported that both arsphenamin and neoarsphenamin were curative for mice inoculated with *B. erysipelatis suis* (*Erysipelothrix Rosenbach*) of swine erysipelas. Baum and Harrenheiser,[74] however, had previously reported that they were unable to confirm Bierbaum's claim that these arsenicals were bactericidal for this organism *in vitro* and that their *in vivo* tests likewise yielded negative results. Kolle and Schlossberger,[75] however, have recently repeated some of this work with dyes and various arsenicals and found that arsphenamin and neoarsphenamin as well as their metallic salts, were effective when used very early in the disease and in doses which approached the limit of toleration.

Chemistry of Mercurial, Creosote and Hexamethylamin Compounds in Relation to the Chemotherapy of Bacterial Diseases.—The chemistry of the compounds discussed in the preceding sections bears an important and fundamental relationship to this whole subject of selective bacteriostasis *in vitro* and disinfection *in vivo*, but unfortunately we do not possess at present much definite information on this question of chemical constitution in relation to chemical disinfection in bacterial diseases.

Insofar as the compounds of *mercury, copper, gold, silver and other heavy metals* are concerned, I believe that bactericidal activity both *in vitro* and *in vivo* is largely a matter of disassociation of the metallic ions. Pilcher and Sollmann[76] have expressed this view in relation to the organic protein and colloidal silver compounds and they have found that blood does not hinder the bactericidal properties of the ionized, inorganic silver salts quite as much as normal saline solution, but hinders the colloidal silver-protein compounds considerably more by acting as a protective colloid and thereby securing a finer suspension of silver chlorid facilitating its solutions or by combining with silver and thereby diminishing its ionization. I have discussed this subject of ionization, however, more fully in Chapter LIII in relation to the mechanism of the toxic effects of mercury for body cells and of curative activity in syphilis; I believe that the same principles apply to the organic and inorganic compounds of other metals. However, it cannot be stated that the toxicity and bactericidal properties of these compounds is solely one of dissociation or ionization of the metallic ions. Abelin,[77] for example, states that the toxic effects of the mercurials can be diminished by the introduction of a sulpho-or sulphamino-group or through double carbon connection of mercury. The relation of the sulpho-group is particularly interesting and important and probably possesses a relation to the toxicity of the organic arsenicals as well, as discussed in Chapter XLVI.

Schranth and Schoeller[78] have observed that the disinfectant powers of organic mercury compounds were increased by substitution of the less acid phenolic hydroxyl for the carboxyl. They also showed that, of the three *isomeric mercuriated* cresols, the meta-derivative is the most potent, while the ortho-hydroxy-mercuri-phenoxid is more effective than the para-compound, and also that the entrance of a second hydroxymercuric group increases bactericidal activity.

In a study of the tuberculocidal activity of a series of new organic preparations of mercury with various phenolic and anilin compounds, DeWitt[79] has also obtained some interesting data bearing upon the relation of chemical constitution to bactericidal activity. Bechhold and Ehrlich[80] and Bechhold[81] in a study of creosote compounds have emphasized the fact that the effect of a given chemical on one species of bacteria may not be duplicated with another species, and hence general laws covering the influence of various modifications of a substance on its bactericidal action cannot bededuced from limited observations. Thus it was found that the introduction of halogen atoms into phenols increases the disinfectant action somewhat in proportion to the number of added halogens.

But it is hardly profitable at present to review in this treatise the numerous opinions expressed from time to time on the relation of chemical constitution to disinfectant activity of the various compounds already listed or described in the present chapter, but another example of what persistent and good work has done in this important field may be found in the papers of Jacobs and his colleagues in their studies with the quartenary salts of hexamethylenetetramin.

Toxicity of the Mercurial Compounds in Relation to the Chemotherapy of Bacterial Diseases.—In regard to the treatment of bacterial diseases, only the mercurial compounds require much consideration from the standpoint of toxicity and especially mercurochrome, because it is the only compound of the kinds discussed in the chapter being generally employed in addition to the organic arsenicals like arsphenamin, neoarsphenamin and sodium cacodylate.

The toxicology and pharmacology of mercury and the arsenicals are, however, discussed with more detail in succeeding chapters and here I shall but briefly summarize the toxic doses of mercurophen and mercurochrome for the lower animals by intravenous injection and the effects produced in human beings by the intravenous injection of these compounds.

Toxicity of Mercurophen.—As a general rule, the maximum tolerated dose of *mercurophen* for white rats by intravenous injection is about 0.006 to 0.008 gm. per kilo of weight. The compound is, therefore, slightly less toxic than mercuric chlorid which has a maximum tolerated dose of about 0.002 to 0.004 gm. per kilo. I have injected mercurophen intravenously in adults in dose of 0.06 gm. or 12 to 15 c.c. of a 0.5-per cent., solution, corresponding approximately to one-eighth of the maximum tolerated dose without reactions; possibly larger doses would produce reactions similar to those produced by mercurochrome, but I have used the drug only in the treatment of syphilis as will be described in succeeding chapters, and general reactions have not been produced by doses of this size although the gingival tenderness and diarrhea of mercurialization have sometimes occurred.

I have already mentioned the toxicity of mercurophen by subthecal injection in the treatment of experimental meningitis. Special mention may be made, however, of the fact that solutions of this compound are particularly well borne by the skin and that individuals possessing a hypersensitiveness to bichlorid of mercury with the production of dermatitis have used it without such reactions. Dr. Schamberg, Dr. Raiziss and I have also observed that it is well borne by mucous membranes and may be employed in dilutions of 1:2000 to 1:4000 for the treatment of conjunctivitis and vaginitis although somewhat higher dilutions, like 1:5000 to 1:7000, should be used for irrigation of the male urethra to avoid burning and other symptoms of irritation.

Toxicity of Mercurochrome.—Mercurochrome is much less toxic due to its lower content of mercury and slower dissociation. White rats ordinarily

stand doses as high as 0.030 to 0.040 gm. per kilo without apparent harm although rabbits and dogs are more susceptible. Young, White and Swartz state that they have noticed a variation in the amount of the drug that rabbits and dogs could tolerate. Ten mg. per kilogram invariably killed rabbits in twenty-four hours, and no gross lesions were found at necropsy. Rabbits receiving 5 mg. per kilogram showed a decrease in phenolsulphonephthalein output and an albuminuria that lasted about a week. Dogs tolerated 10 mg. per kilogram very well with no evidence of discomfort or illness. In each instance there was produced an albuminuria without casts and a temporary reduction in phenolsulphonephthalein output but no rise in blood urea. The albuminuria persisted about five days. At no time were casts found. The phenolsulphonephthalein output returned to normal with the disappearance of the albuminuria. No evidence of kidney damage was found at necropsy on animals killed at the end of the experiments. With this toxic limit established no harm is to be expected from the small amount of the drug that may be absorbed when used locally in the genito-urinary tract.

One-per cent. solution mercurochrome-220 gave no evidence of irritating qualities when used in the conjunctival sac of rabbits. Solutions of this drug in strength from 0.1 to 5 per cent., have been used in the human genito-urinary tract as a local antiseptic. In the kidney pelvis a 1-per cent. solution was used. This was slowly injected through the ureteral catheter, the catheter was plugged, and the solution was retained for five minutes. There has been no sign of irritation or reaction following its use. This procedure has been carried out three times in one week in some instances. In the urethra, a 5-per cent. solution caused only temporary burning when retained five minutes, and a number of cases of acute urethritis have been treated by the use of 1-per cent. solution injected 4 times a day, the solution being retained five minutes at each injection. There has been no irritation beyond occasional temporary smarting.

Hill and Bidgood[82] have recently made a special study of the effects of mercurochrome on the kidneys of dogs reaching the conclusion that the intravenous injection of this compound "causes a mild reaction in the kidney which is directly proportional to the dose given. There is no actual destruction of tubular epithelium from doses as high as 7.5 mg. per kilo of body-weight, but with 10 mg. per kilo there is definite renal damage, so that it would be unwise to use so large a dose clinically."

Miss Rule and I, in our experiments upon the chemotherapy of experimetal pneumococcocus and streptococcus meningitis of dogs, rabbits, guinea-pigs and rats, have observed that the maximum tolerated dose of mercurochrome by subthecal injection is between 0.0001 and 0.0002 gm. per kilo. Injected intracisternally a dose of 0.0001 gm. per kilo is always well borne while a dose of 0.001 gm. frequently proves fatal in thirty-six hours. As a general rule, 0.0001 gm. per kilo can be injected daily for six or more doses, but 0.0002 gm. per kilo is generally fatal after about 3 or 4 daily injections.

Mercurochrome-220 soluble has been prepared for intravenous administration and is generally injected in dose of 0.005 gm. per kilo of weight dissolved in hot distilled water. The solution does not require heating, since it is rapidly self-sterilizing. This dose corresponds to about 0.3 gm. or 5 grains for an adult weighing 130 to 140 pounds and is generally administered in a 1-per cent. solution (25 to 30 c.c.) *slowly* injected. Smaller doses like 10 to 15 c.c. of a 1-per cent. solution, may produce practically no reaction at all beyond increased pulse rate and flushing, but the larger dose is almost sure to produce a severe diarrhea in one-half to six hours and the stools are likely to be red in color due to fluorescein sometimes mistaken for blood; the urine

will also be red in color. The patient may complain of nausea and vomiting, which also may be colored red. A rather severe chill is produced, lasting from ten minutes to an hour but usually not more than twenty minutes, and the temperature is increased by one to three degrees with increased pulse rate. As a general rule, these reactions subside in a few hours.

I have never seen nor heard of a fatal acute reaction of this kind but several individuals have succumbed to the later toxic effects of mercurochrome. I know of several fatalities due to acute nephritis with almost total suppression of urine. Mercurochrome should not be used promiscuously or too freely, and especially if nephritis is present, but I shall discuss this matter at more length in Chapter VIII devoted to the practical chemotherapeutic management of bacterial diseases.

Mechanism of the Curative Activity of the Heavy Metals in Bacterial Diseases.—Unfortunately, but very little has been determined regarding the mechanism of the antibacterial properties *in vivo* of the compounds of mercury, gold, silver, copper, arsenic, creosote, etc., discussed in this chapter. Whatever direct bactericidal effects they may have *in vivo* would appear to be due to the metallic ions after dissociation of the compounds and insofar as the mercurials are concerned, the subject is discussed at length in Chapter XXIV, dealing with their antispirochetal properties. Whether or not the mechanism of curative activity of the mercurials is the same in bacterial diseases as in syphilis cannot be stated; but it would appear that the ions of mercury have a combining affinity for *Sp. pallida* or in some other way are most toxic for these parasites.

It has been frequently suggested that the acute mercurochrome reaction and its curative effects in acute bacterial infections is due to a non-specific protein reaction ascribed to the effects of a new compound of mercury with the plasma proteins. It is true that the reaction bears some resemblance to the effects produced by the intravenous injection of vaccines, peptones and other foreign proteins, but the mercurochrome reaction in my opinion is a colloidal phenomenon and similar in its mechanism to the anaphylactoid reactions produced by the intravenous injection of variety of colloidal preparations of the heavy metals, arsenic, various proteins, etc. According to the results of my experiments with rabbits, the intravenous injection of 0.005 gm. of mercurochrome per kilo usually produces a definite total increase of the leukocytes, chiefly due to an absolute increase of the polymorphonuclear neutrophils and these changes usually occur with an hour after injection and persist for several hours reaching pre-injection proportions at the end of twenty-four hours. It is not improbable but that these leukocytic changes exert an antibacterial influence. Dr. Ling has studied for me the effects of this amount of mercurochrome upon the proteases of the plasma of rabbits, with the following results, expressed in terms of the number of milligrams of on-coagulable nitrogen per 100 c.c. of serum after forty-eight hours' digestion:

	Rabbit 1	Rabbit 2	Average
Before injection	4.2	1.4	2.8
4 hours after injection	2.8	1.4	1.1
24 hours after injection	1.4	0.7	1 0

It will be observed, therefore, that mercurochrome intravenously in rabbits causes a slight drop in the proteolytic power of the serum, so that it is probably not permissible to ascribe any material part of its antibacterial effects *in vivo* to a mobilization or increase of the serum proteases.

In truth we do not know how the colloidal reactions are produced or how bacteria in the blood are destroyed by the mercurials and other compounds mentioned in this chapter; these are still very obscure problems offering great difficulties in elucidation, but warranting and urgently demanding investigation for the purpose of placing the chemotherapy of the bacterial diseases upon a more scientific basis and removing it from the realm of its present state of almost pure empiricism.

Compounds of Mercury, Arsenic and Other Substances in the Chemotherapy of Bacterial Diseases of Man.—It will be apparent from the preceding review of the chemotherapy of bacterial diseases with compounds of mercury, gold, copper, silver, arsenic, creosote, etc., that the subject is still largely if not almost entirely, in the experimental stage. But enough has been done to show that some bacteria may be removed from the blood stream at least by some of these compounds, although there is much less evidence of curative activity in localized bacterial infections of the fixed tissues.

Practically the only compounds of this group used in the treatment of bacterial diseases of man are the arsphenamins in anthrax and streptococcus infections, creosote and guaiacol derivatives in tuberculosis and associated secondary infections, the salicylates in acute rheumatic fever, sodium cacodylate in streptococcus endocarditis and mercurochrome in acute streptococcus, staphylococcus and *B. coli* infections. Of course the colloidal silver-protein and various mercurial compounds have also been used in the treatment of localized infections by topical application and this is likewise a form of chemotherapy, but, aside from the examples just mentioned, we have as yet made but little advance in the chemotherapy of systemic infections of human beings with these compounds.

But encouraging results have been obtained and I firmly believe that the whole field of the chemotherapy of bacterial diseases offers much hope for success and no one will deny the importance of continuing investigation in so important a field and especially since the specific treatment of acute and chronic bacterial diseases by biological substances as serums, vaccines and similar agents so frequently fail to produce beneficial results. But the present state of the value of these compounds in the treatment of bacterial diseases of man is a large subject and I shall discuss in the succeeding chapter what chemotherapy has to offer.

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CHAPTER VIII

CHEMOTHERAPY OF BACTERIAL DISEASES OF MAN AND THE LOWER ANIMALS

In this chapter it is my purpose to summarize briefly what may be designated as the practical applications of chemotherapy in the bacterial diseases. The subject, however, is still in the infancy of its development and while some progress has been made, we do not possess as yet chemical agents for the disinfection of the body of bacteria comparable in efficiency to arsphenamin and its congeners for the treatment of syphilis, quinin for malaria and other remedies like tartar emetic, ipecac, etc., for various protozoan and metazoan diseases. But chemotherapeutic research has resulted in placing at our disposal additional remedies for combating infection, not as substitutes for our usual methods, but rather as adjuvants or aids capable, when properly employed, of improving the treatment of a number of bacterial diseases. I have already discussed in Chapter III some of the more important principles concerned and it is particularly important to bear in mind the influence of the pathological tissue changes of the various acute and chronic infections, the importance of surgical drainage and the advisability in some diseases of combining serum and other forms of biologic therapy with chemotherapy in order to obtain the best results.

Chemotherapy of Wounds, Sinuses and Ulcers.—The treatment of infected wounds with antiseptics has long held a place of great interest in surgical practice. Before the discoveries of Lister one is impressed with the frequency with which wines and balsamics were incorporated into various mixtures for application to wounds, and the good effects were probably due to the antiseptic properties of alcohol. With the work of Lister and the discovery of the pyogenic bacteria, interest in this subject was greatly renewed, although attempts to disinfect wounds were frequently defeated by the use of too toxic and too irritating chemical substances lacking in penetrability and subject to sharp reduction in bactericidal activity by contact with the proteins and the salts of the polybasic acids of necrotic muscles, etc. But the idea of preventing infection or affording aid in the recovery from infections of wounds by the application of disinfectants, has never been entirely abandoned and during the World War interest in the subject was greatly renewed and especially by the work of Carrel and Dakin and their associates with antiseptics of the chlorin group.

It is not usually a difficult matter to determine by bacteriological examinations the kind or kinds of bacteria responsible for suppuration in a wound, although special methods are required for the anaërobes. As a general rule, both Gram-positive and Gram-negative organisms are present in chronic infections and the former are nearly always more numerous and important than the latter, since the Gram-positives include such common aërobes as the streptococci, staphylococci and diphtheroid bacilli, as well as the anaërobes like *B. tetanus*, *B. edematis maligni* and other spore bearing organisms of this group so important in wounds contaminated with dirt. The Gram-negatives are usually *B. coli*, *B. pyocyaneus* and *B. proteus* of much lesser importance. But in the chemotherapy of wounds and sinuses, due consideration must be given to both groups and preference given to a disinfectant or combination of disinfectants known to possess a sufficiently high bactericidal and bacteriostatic activity for spore bearing and non-spore bearing. Gram-positive and Gram-negative, aërobic and anaërobic organisms.

As is well known, wounds do not usually show bacteria in either smears or cultures for at least four to twelve hours and in fresh wounds the surgeon must exercise his judgment on the question of probable bacterial contamination according to the nature and location of the injury; but where it is at all likely that infection is present, disinfection should constitute a very important part of treatment and a subject worthy of close attention from the standpoint of selection of disinfectant and the manner of its application.

Naturally enough, a rather large group of substances and a variety of methods for application have been advocated and indeed, there is by no means a uniformity of either opinion or practice among surgeons upon these questions. It would appear, however, that the choice of antiseptic and the method of its application should be influenced by the conditions and circumstances surrounding individual cases and that no one antiseptic or method is applicable to all wounds. As repeatedly stated, the antiseptic treatment of fresh and suppurating wounds can never be a substitute for drainage and well-established surgical principles in general, but I believe that its possibilities have been generally under estimated as an adjuvant to these. The subject is indeed a very large and comprehensive one, but I shall attempt to summarize briefly here in a practical manner the purely antiseptic or chemotherapeutic phases, without touching upon the surgical and technical aspects so well covered by Carrel and Dehelby[1] and standard works on surgery in general.

1. In small *fresh wounds* which do not require irrigation, the instillation of a 2-per cent. solution of dichloramin-T (sodium toluene sulphondichloramin) in sterile paraffin oil may prove effectual in preventing suppuration and constitutes an excellent first dressing. Thorough cleansing and instillation with a 1:500 solution of mercurochrome in water is likewise recommended. In some cases a paste is to be preferred for furnishing an antiseptic over a longer period of time and I have advised the following 1:500 paste of mercurochrome during the past few years because of its high bactericidal activity, penetrability and soothing blandness: mercurochrome 0.4 gm.; sterile almond or paraffin oil 100 c.c. and bismuth subgallate, 100 gms.

In large fresh wounds which will probably suppurate, and especially those involving bones and joints, it would appear advisable to provide at once for irrigation with Dakin's 0.5-per cent. solution of neutral sodium hypochlorite solution or a 1- to 2-per cent. solution of chloramin-T (sodium toluene sulphonchloramid). Even though the wound may not be sufficiently extensive to demand a series of Carrel tubes, irrigation at two to three hour intervals through a single tube may suffice if the solution is brought into contact with all parts of the wound. Acriflavin gauze may be employed as an antiseptic dressing and particularly since it is odorless, non toxic, does not inhibit phagocytosis or otherwise injure the tissues. It is true that it stains the linen, hands, etc., but these stains are removable by any good soap or by cotton dipped in acid alcohol (3 per cent. hydrochloric acid in alcohol); the formation of the yellow pellicle on the surface of the wound in six to seven days may be sometimes overcome by using normal or hypertonic saline solution.

2. As so clearly demonstrated by Carrel and his colleagues, it is possible to disinfect *suppurating wounds* if provision is made for bringing a properly selected disinfectant into frequent and intimate contact with the tissues. The working out of a method by Carrel whereby this may be accomplished constitutes, in my opinion, one of the triumphs of modern surgery and, even though there may be a difference of opinion on the question of whether sterilization may be carried to the point where it is safe to sew up some

wounds, yet there can be no difference of opinion on the advisability of preventing, limiting or stopping suppuration by disinfectants which do not hinder the processes of healing or add to the dangers of secondary hemorrhage. Insofar as the disinfectant is concerned, one may employ Dakin's solution provided the reaction is properly adjusted to guard against vascular ulceration by free alkali; a 1-per cent. solution of chloramin-T exerts more prolonged antiseptic action, is less toxic and irritating and is to be preferred when acting in the presence of much blood. Or what is better still is to use Dakin's solution for the first day or two followed by the latter. I have also added mercurochrome* to both of these solutions in the proportion of 1 gram to 2000 c.c. to increase bactericidal activity when it was desired to bring about as quick sterilization as possible and especially in streptococcus, gas and malignant edema infections; this gives a 1:2000 solution, but occasional irrigations are not at all dangerous from the standpoint of absorption of toxic amounts of mercury even in large wounds.

Churchman[2] has advocated a mixture of 1 gram each of gentian-violet and neutral acriflavin in 2000 c.c. of sterile distilled water. The former is for the destruction of Gram-positive and the latter for Gram-negative organisms. The solution is of a deep plum color and is objectionable from the standpoint of staining the dressings and bedclothes unless considerable care is exercised, but it is absolutely non-toxic and possesses a high degree of bacteriostatic and bactericidal activity in the presence of pus. Of course, mixtures of these dyes with Dakin's solution cannot be used because of immediate precipitation; neither can they be mixed with mercurochrome for the same reason, but the dyes alone are highly bactericidal and may be used as a substitute for the chlorin compounds for irrigation purposes.

3. Mention may be made of the disinfection of that large group of infected wounds and *sinuses* following operations upon infected tissues requiring drainage. Needless to state, the presence of a suture, necrotic bone or other foreign body acting as the focus of continued suppuration requires surgical removal, but what may be done by appropriate chemotherapeutic measures in the removal of the secondary infection which may be long continued and persistent if the general and local resistance of the patient is greatly reduced? In these I believe that much can be done as an adjuvant to surgery by disinfection and autogenous vaccine therapy. The indications are to use antiseptics in such form that their activity will be maintained over a long period of time in order to reduce to a minimum the degree of handling in order not to disturb granulation and healing. The disinfectant must also be one least likely to prove toxic for fibroblasts and it should not retard phagocytosis or neutralize other immunological processes. For this purpose a 2-per cent. solution of dichloramin-T in sterile paraffin or almond oil may be employed and I have also used the paste of mercurochrome described above; also the following mixture which is thin enough to be poured into a wound or injected into a sinus for the purpose of reaching all parts:

Gentian-violet Neutral acriflavin	.aa	0.5 gm. (gr. viiss)
Bismuth subgallate		300.0 gm. (℥ ixss)
Almond oil		300.0 c.c. (f℥ x)

This gives approximately a 1:1200 suspension of the two bactericides suitable for the treatment of wounds caused by either Gram-positive or Gram-negative organisms or a mixture of the two, as is so commonly found

* Throughout I am using the word "mercurochrome" as a synonym for "mercurochrome-220 soluble." The latter is the sodium salt of mercurochrome but the free acid is not employed and I believe the shorter term is just as good.

in smears and cultures. The paste is not thick enough to retard drainage, the tissues are gradually stained by the dyes and the more deeply situated organisms are thereby reached and gradually destroyed.

4. Finally the matter of the disinfection of *ulcers* may properly command our attention. Needless to state, syphilitic ulcers respond promptly to appropriate systemic treatment and the local lesions so frequently caused by the breaking down of gummas do not require any special consideration. But the ulcers of the aged due primarily to vascular sclerosis plus bacterial infection, as well as the ulcers of diabetes, tuberculosis, and even of epithelioma, may require something in the way of local disinfection for the purpose of checking tissue necrosis as much as possible while other appropriate therapeutic measures are being employed. As a general rule, bacteriological examinations show the presence of staphylococci, *B. pyocyaneus*, *B. coli*, *B. proteus* and diphtheroid bacilli but pathogenic anaërobes are generally absent. A great number of various local applications have been advocated for the primary purposes of disinfecting the tissues and stimulating the processes of repair. Most, however, are lacking in penetrability and fail to reach the organisms situated in the granulation tissue and deeper recesses of the lesions. As a general rule, only such preparations are to be preferred in order that disinfectant activity may be prolonged and granulations protected, but these should not interfere with drainage and I believe that any of the following are to be recommended: (1) a 1-per cent. solution of dichloramin T-in oil; (2) a 1:2000 of mercurochrome in equal parts of almond oil and bismuth subgallate; (3) A 1:2000 of acriflavin and gentian-violet in equal parts of almond oil and bismuth. The two last mentioned readily stain and penetrate the tissues and all three are highly bactericidal in the presence of the excretions without being injurious to the granulation tissue or irritating to the tissues.

Chemotherapy of Burns.—From the standpoint of the chemotherapy of burns the chief indication is to reduce infection and scarring to a minimum. At the same time local applications should be anesthetic, like packs of ½ to 1 per cent. novacain, with 10 minims of adrenalin in each ounce, at least for the first day or two, and always bland in order to protect granulation and healing. As a general rule, the surgical removal of devitalized tissue may be deferred for twenty-four to forty-eight-hours for recovery from shock at which time this and the puncturing of vesicles may require attention followed by spraying with 2-per cent. solution of dichloramin-T every three hours.

Many different disinfectant preparations have been advocated, like 1-per cent. solution of picric acid, which is about 5 times more bactericidal than phenol. The well-known carron oil (equal parts of linseed oil and lime water) is soothing and feebly bacteriostatic as a result of its alkalinity. The disinfectant should be especially active for staphylococci and streptococci, particularly the former, and 1:1000 gentian-violet or acriflavin serves this purpose very well, as in the following paste:

Gentian-violet or acriflavin	0.1 gm. (gr. iss)
Bismuth subgallate	50.0 gm. (℥iss)
Almond or paraffin oil	50.00 c.c. (f℥iss)

The addition of 0.5 gm. of butyn renders the paste anesthetic and its application affords prompt relief, which may be required for the first dressing. Mercurochrome in the same dose may be substituted for the dye in the above formula, and owing to its low toxicity there is not usually danger from absorption, except in very extensive applications.

If the burned area is sprayed or painted with one of the paraffin mixtures, it may be well to incorporate a bactericide in order to prevent staphylococcus suppuration of the granulation as much as possible. For this purpose,

however, our choice of disinfectant is quite limited since gentian-violet, acriflavin, mercurochrome, etc., are not soluble in these mixtures. Resorcinol, betanaphthol, oil of eucalyptus, etc., have been incorporated, but their bacteriostatic and bactericidal activities are very slight and I have found dichloramin-T to be much more active. A 2-per cent. mixture is made as follows:

Paraffin (melting point about 56°C.)	67 gm. (℥ij)
White Vaselin	25 gm. (℥vi)
Olive oil	6 c.c. (f℥iss)
Melt by minimum heating and add:	
Dichloramin-T	2 gm. (gr. xxx)

This mixture is highly bactericidal and has a melting point of about 50°C.; when used it should be melted at this minimum temperature and applied to the carefully dried burned area with a soft camel's hair brush or sprayed on with an atomizer, the electrically heated atomizer being especially useful. Bactericidal tests have shown that heating the mixture tends to slightly reduce its activity, probably by driving off a portion of the chlorin, and for this reason only sufficient for one application should be heated at a time as required.

Chemotherapy of Streptococcus Cellulitis, Lymphadenitis, Septicemia and Other Surgical Infections.—The chemotherapy of streptoccocus cellulitis and lymphangitis resolves itself into two phases, namely, disinfection of the local lesions and disinfection of the blood, if septicemia (bacteremia) develops.

Insofar as local measures are concerned, the establishment of adequate drainage of pus with least manipulation of the parts, elevation, and rest are of paramount importance; needless to state, external vigilance is required for the detection of new areas of pus formation requiring drainage, although the incision of merely inflamed tissues and especially glands may not be required. A large number of local antiseptics have been used, but I agree with Ochsner[3] that one of the best for cellulitis is a *saturated* water solution of boric acid; this should be applied hot in large wet dressings and kept warm by electric pads or hot water bottles. As stated by Ochsner, the addition of 95-per cent. alcohol to the boric acid solution in quantities of from 15 to 30 per cent. will add a dehydrating effect and help to make the limbs warm and comfortable, relieving the cold, clammy feeling of which the patient sometimes complains when watery boric acid solution alone is employed. I have also added to the boric acid solution or to the boric acid-alcohol solution, 1 gram of mercurochrome to the liter, which yields a 1:1000 solution of increased bactericidal activity. These solutions do not retard phagocytosis, but appear to aid leukocytic migration and the walling off of infected tissues; they frequently relieve the superficial lymphangitis and afford relief from the persistent pain, which not infrequently is excruciating in character.

If drainage tubes have been introduced, it is well to provide for irrigation at two hour intervals, using a syringe with very gentle pressure, or the Carrel method. I believe that neutral sodium hypochlorite solution (Dakin's solution) or 2-per cent. chloramin-T are best suited for this purpose, although the dressings should be changed frequently enough to keep the boric acid solution in contact with the skin as much as possible.

A blood culture should be made in all cases, as septicemia may be present even in those with relatively mild local lesions. If the blood is found sterile and the local lesions are regressing, nothing further is usually indicated or required. But if the local lesions are progressing, more energetic measures may be required and especially if the blood culture shows the presence of a streptococcus.

My first step in systemic therapy is to give 100 c.c. of polyvalent anti-streptococcus serum by intravenous injection with the usual precautions against anaphylactic shock. Since the infusion of physiological saline solution alone is sometimes efficacious for combating the toxemia, I frequently dilute the serum with 200 or 300 c.c. of saline solution and administer it with the usual arsphenamin apparatus. In severe cases a second dose of equal size is given twelve to twenty-four hours later. Even though the blood culture is sterile the administration of the serum is frequently indicated when the local infection is spreading, in order to combat the toxinemia, to prevent bacteremia and to aid in the walling off of the local lesions. I am convinced that virulent streptococci produce aggressin-like toxins capable of retarding phagocytosis; this toxin has long been known as a leukocidin or a poison capable of killing leukocytes, and one effect of the serum may be to neutralize it and to this extent favor leukocytic migration into the lesion and encourage phagocytosis. After one or two intravenous injections have been given, it is sometimes useful to administer subsequent doses of 25 to 50 c.c. by intra-muscular injection while mercurochrome, gentian-violet or some other chemical disinfectant is being administered at intervals by intravenous injection.

If, however, the local lesions continue to spread and especially if bacteremia (septicemia) develops, as indicated by positive blood cultures, more energetic measures will be required. It is good practice, therefore, to conduct a blood culture every day or, at least, every other day, during the acute spreading stages in order to detect and combat this bacteremia as early as possible. Even if the blood remains sterile, but the lymphangitis and suppuration continue to spread despite local and serum treatment, the intravenous injection of a disinfectant may be required.

For this purpose, mercurochrome, gentian-violet, neutral acriflavin, rivanol and arsphenamin have been employed. In my experience, mercurochrome has given best results, the dose being 0.005 gm. per kilogram of weight or about 23 c.c. of a 1-per cent. solution per 100 pounds. The solution is prepared in distilled water, but need not be sterilized, since in this strength it is rapidly self sterilizing. As reported by Young and Hill,[4] mercurochrome has apparently saved some desperately infected individuals and I have had three cases of streptococcus cellulitis and bacteremia whom I believe were saved by this drug administered by slow intravenous injection in the dose mentioned. Sometimes it is necessary to repeat the injection in three or four days and in one of my cases a dose was administered every two days for three doses in succession without apparent injury to the kidneys and with a successful outcome.

Young and Hill have also employed intravenous injections of gentian-violet with some success, although this compound, as well as neutral acriflavin, have been disappointing, in my experience, in cases of acute surgical streptococcus infections. Gentian-violet, however, is less toxic than mercurochrome and reactions usually do not occur at all. The dose is likewise 0.005 gm. per kilogram which corresponds to about 46 c.c. of a 0.5-per cent. solution per 100 pounds. The solution may be prepared in distilled water and does not require sterilization. It is quite safe to repeat the injections daily or every other day for several doses in succession. Shallenberger[5] has reported good results with this dye.

Browning and Cohen[6] and others have advocated intravenous injections of neutral acriflavin in about the same dose as gentian-violet, but Meleney and Zau[7] were quite unsuccessful with the drug in experimental streptococcus injections with rabbits and, as stated above, mercurochrome has proved

more efficacious in my experience. Rivanol, an acridin derivative developed by Morgenroth and his associates, has been recommended in dose of 30 to 50 c.c. of a 1:1000 solution by intravenous injection at daily intervals along with intramuscular injections of 50 c.c. of antistreptococcus serum; I have had no experience with this compound.

Arsphenamin and neoarsphenamin by intravenous injection have also been advocated and especially in streptococcus infections by Birt[8] and others; indeed, quite a large literature has accumulated upon their use in puerperal sepsis and other streptococcus infections, but in my experience they have been less efficacious than either mercurochrome or gentian-violet. If mercurochrome or the dyes are not administered it is always advisable to try the intravenous injection of neoarsphenamin in dose of 0.6 gm. for adults.

The value of blood transfusion is not to be under estimated and especially in prolonged cases with marked anemia, emaciation and other evidences of profound intoxication. If time permits, it may be helpful to immunize a compatible donor by administering subcutaneously 1000 million of the patient's streptococci in a vaccine daily for three doses, transfusion being done five to seven days after the last dose. But to be efficacious a large amount of blood is required. I have had cases, showing no perceptible benefit from 200 to 300 c.c. of blood, show a marked and prompt change in condition following 800 to 1000 c.c. and preferably by a direct method to minimize the degree of post-transfusion reaction.

Whether or not mercurochrome and the dyes administered by intravenous injection are able actually to penetrate the local lesions and disinfect the tissues is not definitely known; from the experiments of Hirschfelder, Malmgren and Creavy[9] this would appear unlikely from the doses mentioned, but yet clinically I have seen the local cellulitis and lymphadenitis apparently relieved in a manner suggestive of some direct local disinfection; however, the evidence of possible disinfection of the blood stream is quite definite and I regard the introduction of mercurochrome and the dyes into the therapy of streptococcus infections as a distinct advance and especially when employed with judiciously selected and administered biological forms of therapy.

Chemotherapy of Staphylococcus Cellulitis, Lymphadenitis, Septicemia and Other Surgical Infections.—While severe staphylococcus infections and especially septicemia (bacteremia) are rarer than streptococcus infections, yet they are more dangerous and especially since our biological forms of therapy are much less efficacious. For these reasons the chemotherapy of staphylococcus infections, and especially of septicemia, commands a great deal of interest and importance.

Local measures providing adequate drainage of pus, rest, elevation, etc., are usually sufficient. As a general rule, the washing out of abscess cavities is neither indicated nor required, although in some cases it would appear advisable when drainage is not as complete as it should be with consequent accumulation of pus, organisms and toxins. For this purpose 1:2000 mercurochrome may be recommended, as likewise 1:2000 acriflavin or gentian-violet. Rivanol in 1:1000 solution has also been employed with apparent success and Rosenstein,[10] Hartel and Kishaling[11] and others have advised its use in washing out abscesses or for direct injection into infected tissues. I have had no experience with it but abscesses do not ordinarily require chemical cleansing if good drainage is provided, and injections of this substance are stated to be quite painful and demanding local anesthesia.

Whenever tubes have been introduced for drainage, occasionally flushing may be indicated and for this purpose plain Dakin's solution or a 2-per cent.

solution of chloramin-T may be used because of their bactericidal activity and dissolving effect upon pus. I sometimes add 0.5 gram of mercurochrome to each liter of either of these solutions, since the resulting 1:2000 solution materially increases bactericidal activity without danger of absorption of toxic amounts of mercury. Local applications of saturated solutions of boric acid are not as efficacious as in streptococcus infections, but yet I believe they constitute one of the best applications available and especially a mixture of 700 c.c. of saturated watery solution of boric acid, 300 c.c. of 95-per cent. alcohol and 0.5 gm. of mercurochrome applied hot and kept hot with an electric pad or water bottles.

If these measures prove insufficient and the lesions spread, more energetic treatment is required. Blood cultures should be made, but with unusual care in the preparation of the skin so that if staphylococci are found and especially *Staphylococcus albus*, there need be no hesitation in concluding that an actual bacteremia is present rather than a culture contaminated by staphylococci from the skin. Eternal vigilance is required for the detection of new abscesses and especially in infections of the mammary glands. Anti-staphylococcus serum is hardly worth while. Young and Hill have reported some remarkable recoveries following the intravenous injection of mercurochrome in dose of approximately 23 c.c. of a 1-per cent. solution per 100 pounds of weight; in a series of five of my cases I believe it proved efficacious in two but was apparently unable to control the blood infection in the other three. The injections may be repeated every two or three days.

Gentian-violet and acriflavin have also been injected intravenously in dose of 0.003 to 0.005 gm. per kilogram of weight in 0.5-per cent. solution; Smith and Casperis[12] have recorded the recovery of a child of fifteen months, suffering with severe staphylococcus infection, ascribed to intravenous injections of gentian-violet and Shallenberger has also used it with apparent success in phlebitis and other staphylococcus infections. In my experience, however, neither gentian-violet nor neutral acriflavin in dose of 0.005 gm. per kilogram have succeeded in materially influencing the blood and tissue infections of three cases, one being due to *Staphylococcus pyogenes albus;* I believe mercurochrome is more likely to produce results and especially disinfection of the blood, although re-infection of the blood may occur, which, in one of my cases, was apparently prevented by the injection of 10 c.c. of 1-per cent. mercurochrome every two days over a period of a week. As in streptococcus infections, I believe that transfusion of 500 to 800 c.c. of blood is sometimes indicated and especially in long standing infections with recurrent abscesses and progressive anemia with emaciation. I am convinced that this procedure has turned the tide to a favorable outcome in two of my cases; infusions of physiological saline solution are also beneficial and much more so in my experience than the administration of saline by rectum by high enema or the drip method.

These measures may not prevent the development of abscesses and as long as the temperature and other symptoms indicate continued infection, a very careful watch should be made for their development. But the employment of mercurochrome, and possibly even of gentian-violet or acriflavin, may materially aid in preventing infection of the blood and even for disinfecting the blood, which thereby reduces the danger of metastatic abscesses in the kidneys and other organs, where surgical drainage cannot be instituted.

Special mention may be made of the value of intravenous injections of mercurochrome in *osteomyelitis* and especially staphylococcus infections. Splendid results are sometimes secured when these are given along with one or more blood transfusions and appropriate local treatment.

Various colloidal preparations of the heavy metals have also been used by intravenous injection in the treatment of staphylococcus infections. Silver compounds have commanded special attention and numerous preparations like "argochrome" (a silver compound of methylene-blue) and "argoflavin" (a silver compound of acriflavin) have been used by Leschke[13] and others on the basis that the rather sharp reactions are of the nature of "protein shock" reactions and beneficial to the patient.

Frouin[14] and others have also administered the salts of tin by mouth in the treatment of staphylococcus infections and the oxid in dose of 0.5 to 1 gm. per day is regarded as curative. My clinical experience with tin oxid, however, has not been satisfactory and bactericidal tests *in vitro* with "stannoxyl," stannous and stannic chlorids, stannic iodid, etc., have shown that they possess but slight bactericidal activity for staphylococci.

Chemotherapy of Puerperal Sepsis.—Puerperal sepsis is usually an infection of the puerperal uterus with streptococci of the hemolytic or non-hemolytic varieties and occasionally with staphylococci, by direct continuity and the lymph channels; the infection rapidly spreads locally and the blood stream becomes infected (septicemia) in the majority of cases, as shown by the results of repeated blood cultures. Obstetricians recognized a *puerperal sapremia*, ascribed to the absorption of the toxins of putrefactive bacteria and purely a local affair which disappears almost immediately upon the institution of drainage; but *puerperal sepsis* designates that extremely dangerous infection of the uterus with streptococci or staphylococci rapidly followed by infection of the blood and so frequently fatal that prompt and intensive treatment is required.

From the standpoint of chemotherapy, the disease resolves itself into attempts to disinfect the endometrium and the blood. I shall not enter upon a discussion of the much mooted subject of the intra-uterine douche, but I am convinced that the establishment of free drainage, the removal of organisms and toxins and the destruction of those that may be reached in the tissues by appropriate douching, are principles of primary importance in this as in other pyogenic infections. While antistreptococcus serum, mercurochrome and gentian-violet or acriflavin by intravenous injection may effectually check and finally remove the bacteremia, yet these compounds are without marked effect upon the local infection of the uterus and adnexa; furthermore, the blood may be continuously infected by the access of organisms from these local lesions and for these reasons I believe that appropriate local disinfection should be a part of the treatment of this dreaded and dreadful disease.

The return flow intra-uterine douche tube is well suited for flushing the uterus at intervals and harm cannot be ascribed to its use when it is properly employed with practically no force in order to guard against an extension of infection by pressure and mechanical injury. But as in the treatment of infected wounds elsewhere, best results follow repeated flushings at intervals of two or three hours and, I believe, that provision should be made for bringing a disinfectant solution into intimate contact with all portions of the uterine cavity and especially the head and horns, by means of the douche tube or even with perforated rubber tubes fashioned after the Carrel tubes, held in place by loosely applied packing and connected with a douche tank or bag. The *vaginal* canal may be likewise loosely packed with gauze impregnated with acriflavin and large vulvar pads provided for the collection of drainage of exudates and the returned disinfectant solution. Objections have been raised against this method on the basis that drainage may be impeded with consequent extension of the infection in the lymphatic and vas-

cular channels, but loose packings which may be renewed once a day cannot be regarded as responsible for these effects. Indeed, a distinct advantage may accrue from loose uterine and vaginal packings by reason of retaining a portion of the disinfectant solution and thereby prolonging its action. Furthermore, vaginal packing facilitates drainage and it should not be forgotten that the vaginal canal itself requires cleansing and disinfection since organisms and toxins from the uterine cavity may undergo absorption from the vagina unless these are removed or destroyed at frequent intervals.

Due care, however, must be exercised in the choice of disinfectant and, out of the large number that have been proposed, I believe that properly prepared neutral Dakin's solution or a 1-per cent. solution of chloramin-T is to be preferred. They are non-toxic, bactericidal, are not ordinarily irritating or painful and tend to dissolve the exudates, which thereby facilitates drainage. Every two or three hours a pint or more may be gently passed through the uterus and vagina, followed by a renewal of the vulvar pads, and twice a day the irrigation may be with a 1:4000 solution of mercurochrome prepared by dissolving 0.5 gram in 2000 c.c. of either the Dakin's or chloramin-T solution. Even though a portion of this solution is retained, and it is hoped that this occurs, there is no danger of the absorption of toxic amounts of mercury, and the bactericidal effects are enhanced with some staining of the endometrium which is likewise desirable as indicating penetration of the disinfectants. Gentian-violet, acriflavin or a mixture of the two in 1:2000 strength may be likewise used, but the above mentioned solutions have been more satisfactory; rivanol in 1:1000 solution has been employed abroad, but I have had no experience with it.

When localization occurs, drainage is required as in any other surgical infection. If in Douglas' pouch, this may be by vaginal puncture; but where there is a definite cornual abscess, infection of the upper portion of the broad ligaments, tubes, ovaries, etc., abdominal drainage may be required and in some cases it is well to provide for the irrigation of such lesions when extension of the infection is to be feared.

A host of substances have been advocated for the treatment of the septicemia by intravenous injection among which may be mentioned mercuric chlorid, formalin, colloidal silver, phenoiodine, arsphenamin, acriflavin, gentian-violet and mercurochrome. Of these, only arsphenamin, the dyes and mercurochrome are worthy of special mention.

Arsphenamin and neoarsphenamin have been used rather extensively and according to some reports with rather brilliant results as reported by Behan,[15] Miller and Chalfont[16] and others. The former in dose of 0.4 gm. and the latter in dose of 0.6 gm., may be tried, but I have not observed any pronounced bactericidal effects and doubt if one is justified in relying upon these products if one or two doses at daily intervals have failed to disinfect the blood as judged by symptomatic improvement and sterile cultures. Kiehne[17] has recently employed a mixture of 0.3 gm. of neoarsphenamin dissolved in 5 or 6 c.c. of water to which is added 2 c.c. of 1:100 mercuric chlorid; this greenish-black mixture is injected intravenously every two or three days and is employed abroad by Linsser in the treatment of syphilis. He has given as many as five injections and believes the results justify employing the method. Piper[18] has introduced the method of treatment with intravenous injections of mercurochrome and with some success; Whitehouse[19] has advocated the intravenous injection of neutral acriflavin in dose of 10 to 15 c.c. of 1:1000 solution morning and evening. Aron[20] has employed intravenous injections of "fulmargin," stated to be a colloidal silver preparation produced by electrical dispersion of the metal and with no protective protein;

Loeser[21] has injected "vuzin" (iso-ethylhydrocuprein) intravenously and for uterine douching, etc.

Antistreptococcus serum has been employed for many years and with occasional brilliant results; I believe that it should always be employed along with chemical disinfectants. Frequent transfusions of small amounts of blood has been advocated by Polak, but I have never observed these to have an appreciable influence upon the septicemia, although in prolonged infections with progressive anemia and emaciation, I believe that the transfusion of 600 to 800 c.c. of blood, preferably by a direct method, has been a life saving measure.

I have seen best results in the treatment of the septicemia by a combination form of treatment with antistreptococcus serum, mercurochrome, the dyes and blood transfusion after the following plan:

1. The *early* intravenous injection of 100 c.c. of polyvalent antistreptococcus serum diluted with 200 c.c. of saline solution and with the usual precautions for the prevention of anaphylactic shock. This dose is sometimes repeated in twelve to eighteen hours and thereafter 50 c.c. is given intramuscularly every other day for three or four more doses, either alone or in association with intravenous injections of mercurochrome or one of the dyes.

2. If a prompt therapeutic result is not obtained with the two doses within forty-eight hours, mercurochrome is injected intravenously in dose of 0.005 gm. per kilogram, which corresponds to 23 c.c. of a 1-per cent. solution per 100 pounds of weight. The injection is given very slowly and since the patient usually needs saline solution, I sometimes dilute this dose with 200 to 300 c.c. of saline and infuse it with an arsphenamin outfit.

3. A blood culture is made twenty-four hours later and, if positive, a second injection of mercurochrome is given (about 3 days after the first). If, however, examination of the urine indicates the presence of kidney irritation, I administer either gentian-violet or neutral acriflavin in dose of 40 c.c. of a 0.05-per cent. solution diluted in some instances with 100 to 200 c.c. of saline solution. Or these dyes may be substituted for by the intravenous injection of 0.4 gm. of arsphenamin (disodium salt) or 0.6 gm. of neoarsphenamin administered in the usual manner. As a general rule, however, mercurochrome can be safely given every three days for three or four doses and is the drug of choice, the injection being stopped with symptomatic improvement and sterile blood cultures.

4. At the end of a week or ten days some patients are benefited by blood transfusion and in my experience best results follow the infusion of 500 to 800 c.c.

5. At the same time, local disinfection is to be instituted as early as possible, according to the plan given above and the usual additional measures of diet, plenty of alcohol, glucose or saline solutions by rectum, etc., should not be overlooked.

Chemotherapy of Bacterial Endocarditis.—Unfortunately, chemotherapy has but little or nothing of proven and definite value to offer at present in the treatment of bacterial endocarditis, so apt to be predisposed by defects to infection usually caused by *Streptococcus viridans* and less commonly by the pneumococcus, gonococcus, staphylococci and *B. influenzæ*. The disease has a high mortality, although when caused by streptococci of low virulence, recovery may ensue, as indicated by the reports of Oille, Detweiler and Graham,[22] Miller,[23] Libman,[24] Biggs[25] and others. Pneumococcus endocarditis, which usually develops towards the end of a lobar pneumonia and most frequently involves the aortic valves, is almost

always fatal. Gonococcus endocarditis is likewise usually an ulcerative infection of the aortic valves and almost always fatal. Staphylococcus endocarditis usually occurs in association with abscesses of the heart muscle in the course of a septicemia as a terminal process. Not infrequently and indeed usually, all forms of acute bacteria endocarditis and especially those caused by streptococci, involve valves already the seat of chronic changes produced by the organism of rheumatic fever, whatever that may be. Streptococcus endocarditis may be acute and fulminating, ending fatally in two or three weeks, but in most cases the disease appears very insidiously with fatigue, evening temperature, etc., and is apt to pursue a prolonged subacute course with progressive ulceration, the production of embolism, anemia and emaciation. It is by all odds the commonest type of bacterial endocarditis and what follows here on the subject of chemotherapy applies mostly to this form of the disease.

Naturally enough, a large number of chemical agents have been tried, but it is hardly worth while reviewing them here because of the negative results. Arsenic, however, has held a prominent place for some years and sodium cacodylate (sodium dimethylarsenate) by intravenous injection is reported by Capps[26] to have probably benefited four of a group of eight cases. Bargen[27] thought that sodium cacodylate apparently had some beneficial influence on the healing of heart-valve infections of rabbits produced experimentally by intravenous injections of streptococci, and suggests that it may stimulate the endothelial cells of blood-vessels and blood sinuses to greater phagocytosis. Libman however, has as yet found no definite results with sodium cacodylate; Biggs states of thirty-five cases treated with the drug in daily dose of three grains by intravenous injection for eight to sixteen weeks, the mortality has been 51 per cent.; in another group not so treated the mortality has been 68 per cent. but that a longer period of observation will be required before the merits of the drug can be finally evaluated in these groups. It is also sometimes stated that large doses of the salicylates (2 to 6 drahms daily) are beneficial.

Blood cultures, and especially repeated cultures of at least 10 to 20 c.c. of blood, usually reveal the presence of the causative organism and particularly the streptococcus, although the patient may not present the usual symptoms of a septicemia, indicating that the bacteremia may be intermittent and due to a more or less mechanical sweeping of organisms from the valvular lesions into the blood without the organisms undergoing much proliferation in the blood. In my experience some focus of primary infection is usually to be found and should always be carefully looked for, especially abscesses at the roots of teeth or forgotten stumps and roots. It may be unwise, however, to extract such teeth at once; I recently saw a patient with *Streptococcus viridans* endocarditis who developed an exceedingly acute exacerbation ending fatally in three days following the extraction of three teeth with streptococcus abscesses. It may be well, therefore, to wait until the bacteremia at least has been checked before probable primary foci are disturbed and even then, I believe it is unwise to extract more than one or two teeth at a time in order that the consequent bacteremia may not be too severe. But a careful and thorough search for primary foci should always be made; in my opinion, the valvular lesions may be regarded as secondary foci with the possibility of being kept active by intermittent bacteremias from a primary focus in the teeth, tonsils, accessory nasal sinuses, etc. In this connection I may state, however, that primary foci must be treated separately from the heart lesion and preferably by extirpation. as no amount of gentian-violet,

mercurochrome or arsenic by intravenous injection appears capable of sterilizing apical abscesses or infected sinuses and tonsils.

In my experience, the intravenous injections of gentian-violet and neutral acriflavin in dose of 0.005 gm. per kilogram of weight, have been without influence upon the disease, even when given every three or four days for 3 or 4 injections. In one case, however, I believe that gentian-violet was the means for removing streptococci from the blood, but I have had no reasons at all for assuming that these dyes influenced favorably the valvular lesions. In my limited experience with three cases, repeated intravenous injections of mercurochrome have been apparently more efficacious insofar as immediate results were concerned; a chill, fever and diarrhea have usually followed the injections, but almost always the temperature has dropped to about normal for the following two or three days with marked improvement in the general condition. But the temperature then rises again and repeated injections are required. Naturally one hesitates to produce in these cases violent reactions of any sort, and especially anything favoring the production of embolism, but in my cases the chills and other symptoms of the mercurochrome reactions were apparently without harm. All three of my cases (streptococcus infections) ultimately succumbed; Young, Hill and Scott[27a] however have recently reported two apparent recoveries in a series of severe cases treated with mercurochrome by intravenous injection.

Antistreptococcus serum has been used extensively but without marked effects. I believe, however, that it may be useful during the acute stages when numerous streptococci are to be found in the blood; blood transfusion may be also beneficial and especially in the later stages when progessive anemia and emaciation develop and all of these methods may be summarized into the following *plan of treatment for Streptococcus endocarditis:*

1. During the acute stage marked by streptococcus bacteremia, 100 c.c. of antistreptococcus serum may be given intravenously with the usual precautions against anaphylactic shock. This dose may be repeated in forty-eight hours and thereafter 50 c.c. may be given by intramuscular injection every three or four days for an additional three doses. Serum sickness is almost sure to develop in a week or two, but is readily relieved by cautious doses of adrenalin (usually 0.5 c.c. of 1:1000 subcutaneously) and local applications. If bacteremia is not present, it is hardly worth while administering serum; likewise, in the subacute and chronic stages serum may be omitted, but in the acute early stages I believe it is worth while, even though the first blood culture may prove to be sterile.

2. Mercurochrome may be given instead of the serum or following the serum. The dose may be slightly reduced below the usual, like 15 c.c. of a 1-per cent. solution per 100 pounds; a second dose may be given four or five days later and usually in dose of 23 c.c. of a 1-per cent. solution per 100 pounds. As a general rule, the temperature drops within twenty-four hours after each injection, but generally rises again within three or four days when further injections are required. I have given one case as many as 6 injections without apparent harm to the kidneys, but one naturally hesitates in giving repeated injections of mercurochrome. Two doses, however, at intervals of three or four days may be regarded as quite safe. At this time another blood culture should be made. If streptococci are found, gentian-violet may now be injected intravenously in dose of about 30 c.c. of a 0.5-per cent. solution per 100 pounds; in one of my cases, an alarming cardiac depression followed but with recovery in a few minutes and no further reaction. These measures usually suffice for disinfecting the blood, but probably have but little or no disinfectant influence upon the valvular lesion. If, however,

measures of this sort keep the blood sterile, life is prolonged with increasing chances of gradual recovery and alternate injections of mercurochrome and gentian-violet may serve this purpose and are worthy of trial.

3. Insofar as the valvular lesions are concerned, it may be that healing is encouraged by the intravenous injection of arsenic and especially since some observers believe that this substance possesses dynamogenic effects, that is, the property of promoting fibrosis; furthermore, arsenic may promote phagocytosis by endothelial cells and clasmatocytes, as suggested by the work of Bargen. For these reasons, I believe that it is well to inject sodium cacodylate intravenously every other day in dose of about three grains for an adult, although, frankly, I have not yet observed that this drug produced any material therapeutic effect. This dose corresponds to approximately 0.2 gm. per 140 pounds, which is a safe amount, since the single maximum tolerated dose for the lower animals is at least 40 to 50 times this dose per kilogram of weight (for rats and rabbits from 0.15 to 0.2 gm. per kilogram). Or neoarsphenamin in dose of 0.3 gm. dissolved in 5 c.c. of sterile water may be injected intravenously twice a week, purely for the purpose of eliciting these possible effects of arsenic upon healing and phagocytosis and with no hope of securing a direct disinfecting effect. It may be also worth while to give an occasional injection of mercurochrome in dose of 10 c.c. of a 1-per cent. solution and I have followed up the first two or three large doses with several of these small doses at intervals of three days with the hope of favorably influencing the valvular lesions. What with injections of mercurochrome, gentian-violet and the arsenicals, the treatment is hard pushed but the gravity of the disease warrants and demands energetic measures in most cases; of course, good clinical judgment must be exercised in the choice of these agents, their doses and intervals of administration and the condition of the patient's enunctories must be estimated in a careful manner with special reference to the condition of the kidneys.

4. If, in the meantime, the streptococcus recovered in blood cultures or abscesses of the teeth is prepared in a vaccine, it may be worth while to give the patient a subcutaneous injection once a week, starting with a small dose like 50 million and gradually increasing. It is also a good plan to immunize a compatible blood donor, as the transfusion of 500 c.c. of blood, preferably by a direct method, not infrequently benefits the patient and is especially valuable for the support it gives in tiding the patient over a crisis or a prolonged septic infection. I have generally given my patients three or more transfusions for these effects, even though there is little or no hope of sterilizing the local valvular lesions by these means which must heal by natural processes aided somewhat by the measures here summarized.

Why it is that so little can be done for these cases is difficult to state. Off hand, one would suppose that the presence of gentian-violet, mercurochrome, etc., in the blood would have some sterilizing influence upon the lesions and especially by direct contact with the open ulcers even though the bactericidal concentration in the blood is only a matter of an hour or two following each injection; but the constant flapping of the valves deprives them of functional rest, which is the most important single factor required for recovery from inflammation. Apparently only the mildest of cases produced by streptococci of reduced virulence have a fair chance of ultimate recovery, although the disease may run a course of months or years. But if we could influence it enough to prevent ulceration, the changes of recovery are increased and I believe that every case should be given this chance despite the gloomy prognosis.

I have had no experience with mercurochrome, gentian-violet or acriflavin in the treatment of pneumococcus, gonococcus and staphylococcus endocarditis, but believe that treatment with these compounds in association with blood transfusions may be worthy of trial as a substitute for folding our hands and leaving the patient to his fate.

Chemotherapy of Pneumonia.—Needless to state, the chemotherapy of pneumococcus lobar pneumonia, so well designated the "captain of the men of death," has deservedly attracted a great deal of attention and, contrary to usual impressions, I believe that it has already accomplished a good deal and is worthy of a place in the modern treatment of this dreaded disease along with functional, regulatory, symptomatic and biologic therapy. Certainly there is no short cut specific treatment of pneumonia and the institution of a dietetic-hygienic regime, the expectant treatment of symptoms like cough, pain, dyspnea, insomnia and abdominal distention, as well as of the complications like severe toxemia, circulatory failure, jaundice, collapse at the crisis, profound asthenia, etc., demand appropriate treatment, in addition to and aside from what may be accomplished by specific biologic or chemotherapeutic measures.

Possibly too much has been expected of chemotherapy in this disease in the way of spectacular results in the form of immediate crises and no mortality. The most that can be expected is the destruction of pneumococci in the affected portions of the lung, to confine the process to the lobe involved, and to prevent the occurrence of empyema and septicemia, as well as complications resulting therefrom; in brief, to prevent direct or indirect extension of the pneumonia from the first focus of the disease. I doubt if any of our present chemotherapeutic measures are capable of penetrating and sterilizing the exudates of a pneumonic lobe, but I believe that there is considerable evidence in support of the hope that we may and do raise the pneumococcidal activity of the blood and lymph to such an extent that bacteremia and septicemia are corrected and prevented which materially aids in confining the infection to the lobe or lobes primarily involved and thereby preventing the successive involvement of fresh tissues with consequently a much greater chance of survival for the patient.

As reviewed in Chapter VI, quinin compounds including optochin base and ethylhydrocuprein hydrochlorid, have commanded most attention from the standpoint of chemotherapy and deservedly so, in view of their extremely high and somewhat selective pneumococcidal activity *in vitro* and encouraging results in experimental pneumococcus infections of the lower animals. Many excellent clinicians, including Da Costa, Aubrecht, Gibson, Solis-Cohen and others, have long believed that quinin exerts a favorable influence, and more recently Moore and Chesney,[28] Jakbo,[29] Schneider[30] and others have corroborated these views with optochin, the rather extensive literature being lately summarized by Cahn-Bronner.[31]

Unfortunately, optochin is capable of producing a toxic amblyopia in about 4 to 5 per cent. of cases, as reported in the literature, but when given by ingestion, as advised by Mendel, Moore and others, this complication is greatly reduced and may be entirely avoided by proper attention to dosage and intervals of administration. As stated by Solis-Cohen, tolerance for quinin is greatly increased in pneumococcus pneumonia, so that individuals with this disease readily withstand doses which would probably produce marked cinchonism in others. I am personally convinced that the following plan of chemotherapy, which is modified after that described by Solis-Cohen, Cahn-Bronner, Moore and others, is worthy of trial even though its value

cannot be stated in exact terms until more extensive clinical experience has been gained with it.

1. The intramuscular injection of 0.5 gm. quinin hydrochlorid with urethane, dissolved in 5 c.c. and injected as early as possible after the chill; 0.5 gm. of ordinary quinin and urea hydrochlorid dissolved in 2 or 3 c.c. of hot sterile water, may be used instead, it being necessary to attend to the following details in technic in order to avoid excessive local reactions: (1) to use a sterile solution containing no acid, (2) to prepare very carefully the skin and (3) to avoid depositing any of the solution in the subcutaneous tissues. If the fever does not fall within twenty-four hours, the injection is repeated. One then waits forty-eight hours and, if the crisis has not occurred, a third injection is given amounting to 1.5 grams within four days. The pharmacology, toxicology and further directions for injecting are given in Chapter VI.

2. Following these intramuscular injections of quinin, 0.15 gm. (2½ grains) of optochin hydrochlorid (ethylhydrocuprein) is given in capsules every three hours, totalling 1.2 gm. or 20 grains, for the purpose of maintaining the increased bactericidal activity of the blood. If injections of quinin cannot be given, optochin may be used instead on the first day in dose of 0.5 gm., followed every three hours by additional doses of 0.15 gm. each; commencing on the second day, the drug is given in dose of 0.15 gm. every three hours as just described. In either case the treatment is continued for three to nine days according to circumstances, but a careful watch is maintained for dimness of vision, which demands a cessation of optochin administration. Daily opthalmoscopic examinations for retinal ischemia are of distinct help in detecting the earliest evidences of amblyopia.

A careful study of statistics by Cahn-Bronner indicates that the mortality rate was only 6.4 per cent. for the cases treated with quinin, whereas in contemporaneous control cases, even after removing from the statistics patients who had entered the hospital moribund, the mortality was 20 per cent. Among the patients that received quinin injections within the first three days of the disease the mortality was only 5.7 per cent. There was noticed also a marked improvement in the general state of the patient (circulation and respiration). In pneumonias, other than those of pneumococcus origin, it is stated that quinin therapy produced no favorable effects.

As stated by Barker,[32] though even with the use of quinin and optochin the mortality rate in lobar pneumonia is still high, nevertheless the reduction indicated by the statistics given would make it seem worth while to make a trial of quinin therapy, at any rate until sufficiently large numbers of cases have been treated in different epidemics and in different places (with adequate controls) to establish the value or the worthlessness of the remedy.

Ochsner[33] believes that the internal administration of boric acid in dose of 5 grains three times a day is of benefit and recently mercurochrome has been used with apparent success by Freeman and Hoppe[34] and others in dose of 0.005 gm. per kilogram of weight. I believe that mercurochrome is especially worthy of trial in bronchopneumonias in which the etiological agent may be a streptococcus or some organism other than the pneumococcus, over which quinin and optochin have no beneficial influence, but even in severe lobar pneumonias (especially Type II infections) the compound has been apparently successful and especially worthy of trial when new lobes become progressively involved as indicated by a recent review by Young, Hill and Scott[34a] of 22 cases of broncho- and lobar-pneumonia treated with intravenous injections. The temperature sometimes falls within twenty hours after injection with an improvement in the general condition, but two

or three doses may be required at intervals of three to four days. Young and Birkaug have recorded the successful treatment of a case of streptococcus pneumonia by Martyn, who gave an intravenous injection of mercurochrome in dose of 0.005 gm. per kilogram, and in this type of pneumonia, the drug would appear to be especially worthy of trial. In addition to these chemotherapeutic agents, judicious biologic therapy in the form of injections of Type I pneumococcus serum or Huntoon's antibody solution are worthy of consideration and especially the latter in all Type II and III pneumonias.

Finally a brief mention may be made of efforts evolving a method for the disinfection of *pneumococcus carriers*. As shown by Dochez and Avery,[35] Stillman,[36] Sydenstricker and Sutton[37] and others, healthy individuals not in contact with cases of lobar pneumococci do not usually show the presence of the fixed types (I, II and III) of pneumococci in the upper respiratory tract, but among contacts they have been found in 6 to 40 per cent. Bearing in mind the numerous difficulties in disinfecting the mouth of pneumococci even with powerful and more or less specific antipneumococcus agencies, as ethylhydrocuprein and other quinin compounds, it is hardly to be expected that complete destruction of all pneumococci in the mouth and upper air-passages can generally be accomplished by this means, but for use among physicians, nurses and members of a family in intimate contact with persons suffering with lobar pneumonia, the systematic and daily use of washes prepared from 1:100,000 solutions of ethylhydrocuprein hydrochlorid or quinin bisulphate in 1:10 liquor thymolis may serve to destroy virulent pneumococci as they gain access to the mucous membrane of the mouth and upper part of the throat and prevent their proliferation in large numbers. In this manner and among such groups of persons, the systematic use of a mouth-wash of this kind held in the mouth and gargled in the throat for at least a minute twice or three times each day may aid in the prophylaxis of lobar pneumonia, as based upon the experiments conducted by Steinfield and myself.[38] Ethylhydrocuprein hydrochlorid by reason of its superior pneumococcidal properties is to be preferred, but may be substituted by quinin bisulphat or quinin hydrobromid, although these are not as powerful bactericides; solutions of either stronger than 1:10,000 are likely to prove objectionable to most persons. Liquor thymolis in itself appears to aid in the disinfecting process and is well borne in a 1:10 dilution, serving also to disguise to a large extent the bitter taste of ethylhydrocuprein or other cinchonic.

Chemotherapy of Pleuritis and Empyema.—Interest in the possibilities of local disinfection of the pleural cavities infected with pneumococci, streptococci, staphylococci, *B. influenzae,* tubercle bacilli or other organisms, has been greatly increased within the past few years and especially since the great pandemic of influenza in 1918, which was characterized by a high incidence of streptococcus pneumonias and empyemas. Some success has been attained in the chemotherapy of experimentally produced pleuritis among the lower animals and the subject is one of commanding interest and practical importance in the treatment of suppurative pleuritis of human beings.

Chemotherapy of Streptococcus Pleuritis and Empyema.—Experience during the past six years and especially with streptococcus empyema has shown that drainage by rib resection should not be done during the acute stage when the fluid is serous or seropurulent, because there are no, or but few, pleural adhesions and the resulting collapse of a large part of the lung adds materially to the mortality. For example, Rodman,[39] Stone,[40] Moschcowitz,[41] the Empyema Commission[42] and others have shown that too early resection of a rib or other surgical intervention is accompanied by a mortality

of 45 to 65 per cent. It is true that a number of closed methods for drainage have been devised by Mozingo,[43] Harloe,[44] Phillips,[45] Manson,[46] Churchman,[47] and Manson and Churchman have devised special means and apparatus for draining and washing out with Dakin's solution or gentian-violet under closed conditions; but it would appear that simple aspiration followed by the injection of gentian-violet, as advised by Major,[48] may be the method of choice in the treatment of acute empyema and Davis[49] has recently reported excellent results with a method conducted as follows:

"The chest over the fluid area is anesthetized with 0.5 of 1 per cent. novocaine. The aspiration is done with a 50 c.c. Luer syringe connected to the needle by a piece of rubber tubing two to three inches in length fitted with adaptors to fit syringe and needle. This prevents air entering the chest at the time of disconnecting syringe and needle and also is not likely to injure the visceral pleura and the lung by movements of the patient or by connecting and disconnecting the syringe. A nurse or assistant maintains the position of the needle. The fluid is aspirated until the cavity is almost dry, this to be determined by the patient beginning to cough. One precaution we have observed is that during the first aspiration if there is a large amount of fluid, that we did not take out an unnecessarily large amount, although we have never seen any bad results from slow aspiration. The one-half as much aqueous solution of gentian-violet (1:2500) as fluid removed is slowly injected into the cavity. The fluid is warmed to body temperature. By a later work of Churchman[50] we know that if the fluid is warmed above body temperature it has more of a bactericidal value. This fluid is allowed to remain until the next aspiration which should be done from one to five days, depending upon each individual case. During the second and following aspiration a solution of 1:1000 is used."

In subacute and chronic empyema with drainage by rib resection, irrigation with properly prepared neutral Dakin's solution with a free chlorin content of 0.4 to 0.5 per cent. would appear to be the method of choice, according to the results recently reported by Stevens.[51] Apparently the cavity becomes sterile more quickly than if treated by simple drainage without irrigation; the wound is cleansed and the exudate removed, and the excess scar tissue over the surface of the lung is dissolved, allowing the lung to expand and obliterate the cavity.

Stevens has emphasized the necessity of using properly prepared solutions. If they are too alkaline the tissues may be dissolved too quickly and thereby promote hemorrhage and the production of pleuropulmonary fistulas and if these are present at the time of thoracotomy, irrigation should be omitted or delayed; if the chlorin content is below 0.4 per cent., sterilization is faulty or may not occur at all. Hedlbom[52] has also reported favorable results from irrigation with Dakin's solution.

Irrigation every three hours is provided for by means of the Carrel tubes and best results are observed when irrigation is begun at once; thus when irrigation was instituted on the average of 90 days after resection and drainage, it required an average of 73 days for sterilization and an additional 71 days for healing making a total of 144 days; when irrigation was begun immediately after operation the time required for sterilization was 42 days with an additional 57 for healing or a total of about 98 days. Furthermore, the percentage of recurrences was materially decreased as likewise the percentage of thoracoplastic operations for the obliteration of cavities. Failures were ascribed to one of four things: (1) necrosis and infection of the resected rib; (2) a foreign body in the cavity (either a small sequestrum of bone, gauze or a drainage tube); (3) a pleuropulmonary fistula which caused constant reinfection of the sinus, or (4) sacculations and diverticula which could not be reached by the usual methods of irrigation. With these conditions remedied, sterilization was prompt.

Chemotherapy of Pneumococcus Pleuritis and Empyema.—As previously stated, most of the cases treated with simple aspiration and the injection of

gentian-violet or by rib resection, drainage and irrigation with Dakin's solution, were streptococcus empyemas, but the same methods are applicable for the treatment of pneumococcus and staphylococcus infections. Pneumococcus infections apparently respond even more rapidly since these organisms are more readily destroyed than streptococci. In acute pneumococcus empyemas, therefore, requiring the removal of pus, it would appear proper to aspirate during the acute or early stages and to inject gentian-violet as described above. A 1:1000 solution of ethylhydrocuprein hydrochlorid may be tried instead, being used in exactly the same manner and in the same dosage; but in my experience with two cases, apparently best results were secured by injecting a hot mixture of equal parts of 1:1000 gentian-violet and 1:1000 ethylhydrocuprein dissolved in sterile distilled water.

There is no danger of toxic amblyopia from the ethylhydrocuprein, as the amount injected in one day does not exceed 0.05 gm. which is perfectly safe, does not irritate the tissues and very materially increases the rate and degree of pneumococcidal activity. In cases requiring rib resection and drainage, however, preference is to be given to irrigation at three-hour intervals with neutral Dakin's solution, having a chlorin content of 0.4 to 0.5 per cent., although it may be worth while to use 1:1000 ethylhydrocuprein in place of the Dakin's solution once or twice a day for the purpose of increasing the rate of sterilization.

Chemotherapy of Staphylococcus, Tuberculous and Mixed Pleuritis and Empyema.—In staphylococcus empyemas the treatment may be exactly as decribed for streptococcus empyemas, but in any acute case of mixed infection with streptococci or pneumococci with Gram-negative bacilli like *B. friedländer* or *B. influenzae*, etc., it would appear worth while to aspirate and inject equal parts of 1:1000 gentian-violet and 1:1000 neutral acriflavin.

Chemotherapy of Scarlet Fever.—Since scarlet fever is now regarded as caused by a hemolytic streptococcus on the basis of the brilliant investigations of the Dicks, it is entirely likely that an efficient chemotherapy will be developed in time. Numerous reports by Lenzmann,[53] Laques,[54] Klemperer and Worta,[55] v. Boltenstern,[56] Lenzman,[57] Glaser,[58] and others indicate that intravenous injections of arsphenamin or neoarsphenamin may be distinctly worth while in the treatment of severe cases, as it would appear that the temperature is lowered along with a reduction in the incidence of complications and mortality. Since these arsenicals have been reported upon favorably in the treatment of other streptococcus infections and especially puerperal sepsis, they may be worth while using alone or especially in conjunction with the Dick or Dochez serum in the treatment of unusually severe cases. The dose of either for children may be from 0.1 to 0.3 gm. and for adults 0.5 to 0.6 gm. by intravenous injection; apparently arsphenamin (disodium salt) has given better results than neoarsphenamin.

Young and Birkhaug[59] have recently reported the successful treatment of an unusually severe case of scarlet fever complicated with erysipelas and septicemia, by intravenous injections of mercurochrome, and this compound may be quite worthy of trial in severe cases of scarlet fever, especially those showing the presence of large numbers of streptococci in the blood. As shown by Hirschfeller,[60] ethylhydrocuprein has no effect upon either scarlet fever or measles.

Chemotherapy of Meningococcus Meningitis.—Serum therapy has greatly reduced the mortality and incidence of sequelae in meningococcus meningitis and, to the best of my knowledge, chemotherapy has little or nothing to offer at the present time. If a bacteremia is present as detected

by blood cultures, it may be that intravenous injections of mercurochrome or neutral acriflavin may be of aid, but better results are secured by intravenous and intramuscular injections of antimeningococcus serum. Arsphenamin, neoarsphenamin, intramin, soamin, antimony tartrate, hexamethylentetramin (urotropin) and helmitol have been tried by intravenous or oral administration but with generally negative results. Most interest has been commanded by hexamethylentetramin because it is known to be capable of reaching the spinal fluid in meningitis when administered by mouth or intravenous injection, but its bactericidal activity depends upon the liberation of free formaldehyd and this does not occur in the spinal fluid because of its alkaline reaction; clinically the administration of this compound has not produced results even when given intravenously in doses as high as 60 grains for an adult.

Helmitol (hexamethylentetramin anhydromethylene citrate) is said to liberate formaldehyd in an alkaline as well as in acid media and while it possesses some bactericidal effects upon meningococci *in vitro*, yet it would appear impossible to administer sufficient by intravenous injection (60 grains) or by intrathecal injection (10 to 30 grains in an ounce of saline or horse serum) materially to influence meningococcus meningitis or to detect free formaldehyd in the urine or cerebrospinal fluid.

A variety of chemical agents like lysol, phenol, protargol and eusol have been injected intrathecally, but likewise without beneficial results; indeed, these substances may prove distinctly harmful. Thus, Flexner and Amoss[61] have found lysol and protargol harmful in experimental meningococcus infections of guinea-pigs and monkeys on account of their antileukotatic and antiphagocytic properties and since recovery from all forms of meningitis is largely due to phagocytosis, it is very important that nothing is injected capable of paralyzing this important process.

On the other hand, however, the oral administration of helmitol in dose of 15 grains every twenty-four hours is said to keep the cerebrospinal fluid under its influence and its administration may be helpful in the treatment of those fulminant and apparently serum resistant cases of meningococcus meningitis occasionally encountered. Furthermore, the intraspinal injection of neutral acriflavin in dose of 0.0001 gm. per kilogram (0.1 c.c. of 1:1000 per kilogram) mixed with the serum may be worth trying, although in a few cases in which this compound has been injected, the results have not been encouraging.

Chemotherapy of Pneumococcus, Streptococcus and Staphylococcus Meningitis.—Septic meningitis of otitic or nasal origin is one of the most dreaded of diseases since the mortality is about 100 per cent. with ordinary methods of treatment. I have never known streptococcus meningitis to develop without a primary focus of infection in the mastoid, accessory nasal sinuses or as a result of fracture of the skull, but pneumococcus meningitis may develope during lobar pneumonia or occasionally without a discoverable primary focus. Cases of this sort have a better prognosis and while the mortality in general for pneumococcus meningitis has been placed at about 93 per cent., yet I believe that in severe septic leptomeningitis of otitic or nasal origin it is about 100 per cent. when treated in the ordinary manner by spinal puncture and intraspinal injections of immune sera.

As stated in Chapter VI, Idzumi and I were sometimes able to save rabbits with experimental pneumococcus meningitis when ethylhydrocuprein (numoquin) was injected intraspinally within four hours after infection, but never when treatment was instituted after the onset of symptoms. In more recent experiments with streptococcus, pneumococcus and staphylococcus menin-

gitis of rats, rabbits and dogs conducted with the assistance of Miss Rule and Mr. Madden, I have found ethylhydrocuprein, gentian violet, neutral acriflavin, rivanol and mercurochrome ineffective when used alone after the development of symptoms.[61a] In these experiments the compounds were administered intravenously in dose of about 0.005 gm. per kilogram and also by intraventricular and intracisternal injection in doses varying from 0.0001 to 0.0004 gm. per kilogram, corresponding to a dose of 6 to 24 c.c. of 1:1000 solutions per 60 kilograms or approximately 130 pounds of weight. However, dogs infected by intracisternal injections of a Type I pneumococcus and a hemolytic streptococcus were frequently saved after the onset of symptoms by trephining and washing the ventricles to the cisterna magna with warm Ringer's or saline solution.

The diagnosis of septic meningitis of otitic or nasal origin is frequently difficult, that is to say, whether the meningitis is purely localized and aseptic with a favorable prognosis or a spreading and diffuse leptomeningitis. As a general rule spinal puncture is too long delayed and especially because many physicians fear to remove spinal fluid because of the possibility of setting up a meningitis by favoring the localization of organisms in the meninges from the blood. But I am quite sure that this danger is greatly over estimated and that the careful removal of 3 to 5 c.c. of fluid, ample for diagnostic purposes, is without harm.

In mastoid, lateral sinus or nasal accessory sinus infections it is usually an easy matter to discover the organism present in the discharges and it would appear to be good practice to attempt immediately passive immunization against the pneumococcus, if present, by the intravenous injection of 25 to 50 c.c. of Huntoon's antibody solution or against the streptococcus, by the injection of 50 c.c. of polyvalent antistreptococcus serum. Certainly these would appear advisable upon the first appearance of signs of meningitis when one is in doubt whether the infection is or is not localized.

I believe it is also well to attempt disinfection of the primary focus if this is at all possible; in mastoid cases, for example, infected clots may be removed and a rubber tube inserted into the wound for flushing every two or three hours with a mixture of equal parts of 1:1000 gentian violet and acriflavin. Dakin's solution may be used instead of the dyes, but it must be neutral and have available at least 0.4 to 0.5 per cent. chlorin. The disinfectant is readily introduced by means of a syringe attached to the tubing or by a douche bag and the fluid collected in large gauze pads frequently renewed. If the dyes are used it may be advantageous to dissolve them in 10-per cent. saline instead of water, as irrigation with hypertonic solution may reverse lymphatic absorption and promote better drainage. In some cases drainage tubes or silver wire have been introduced into the cisterna pontis through the internal auditory meatus after the destruction of the labyrinth with successful drainage of the localized infection. If these measures are taken before the exudate has become plastic it may be possible to irrigate the basal cisterna with Ringer's solution by the method of Eagleton,[62] needles being placed in the cisterna magna and lumbar region. In sinus thrombosis and phlebitis Eagleton also advises ligation of the internal carotid artery on the affected side to place the parts at rest and facilitate operative procedures. To be effective, measures of this sort must be instituted at an early stage, although Eagleton has abandoned his former method of continuous subarachnoid irrigation.

But I believe drainage is the most important factor of treatment. Dandy[62a] reports the recovery of three cases of septic meningitis by continuous drainage of the cisterna magna; Rainey and Alford[62b] have also recorded

the recovery of two cases of septic meningitis by the institution of continuous spinal drainage by laminectomy and the insertion of a small catheter into the subarachnoid space. Septic meningitis therefore, is to be looked upon as a surgical disease demanding radical measures since a fatal outcome is almost sure by the usual treatment of spinal punctures and the intraspinal injection of immune sera.

But chemo-sero-therapy may be useful as an adjuvant measure. Wolf and Lehman,[63] Rosenow,[64] Cordua[65] and others have reported apparently successful results in the treatment of pneumococcus meningitis by intraspinal injections of ethylhydrocuprein hydrochloride. I have used it in eleven cases, injecting daily or twice daily, 15 c.c. of a 1:500 solution after removal of spinal fluid, but without success insofar as mortality was concerned. It is possible that better results may be secured by injecting Huntoon's pneumococcus antibody solution intraspinally in dose of 15 to 30 c.c. along with subcutaneous or intravenous injections of 25 to 50 c.c. Wieder[65a] observed a good primary result with it and Ervin[65b] has recorded the recovery of a case treated in this manner. I have also observed a beneficial effect in one case. In streptococcus meningitis it may be worth while to inject gentian violet intraspinally in dose of 5 to 20 c.c. of a 1:1000 solution; Royster[66] has reported a case in which life was apparently prolonged by the injection of very small doses varying from 0.08 to 0.2 mg.

But I am sure that serum therapy alone by intravenous or subthecal injection is insufficient and the same may be stated of such chemotherapeutic compounds as ethylhydrocuprein, mercurochrome and the dyes. When administered intravenously they may remove organisms from the blood but do not appear capable of influencing favorably the meningeal infection. Furthermore, it does not appear possible to introduce sufficient by intraspinal, intracisternal or intraventricular injection to influence the mortality. It is necessary to promptly institute drainage along with lavage when possible and employ these other agents as adjuvants, if any success is to be gained at present in the treatment of septic leptomeningitis of otitic or nasal origin.

Chemotherapy of Lethargic Encephalitis and Poliomyelitis.—At present chemotherapy has nothing to offer in the treatment of lethargic encephalitis or acute anterior poliomyelitis. I know of one case of the former disease apparently greatly improved and aided in recovery by the intravenous injection of neutral acriflavin in dose 0.005 gm. per kilogram, but further experience is required before any opinion can be offered regarding its value in this disease. Since it is possible that streptococci may be of etiological importance in both diseases, it may be advisable to try not only acriflavin but mercurochrome by intravenous injection; Visher[67] had recently reported the prompt improvement of two cases of encephalitis, following the intravenous injection of 10 and 8 c.c. respectively, of a 1-per cent. solution of mercurochrome Young, Hill and Scott[67a] also record two cases of acute anterior poliomyelitis treated with mercurochrome by intravenous injection with apparently beneficial results.

Chemotherapy of Typhoid and Paratyphoid Fevers.—Although the early bacteremia and prolonged course of typhoid and the paretyphoid fevers would appear to offer exceptionally good opportunities for chemotherapeutic disinfection of the blood and lymphoid tissues, these diseases have not so far engaged much attention from the standpoint of chemotherapy. In part, this is due to the impossibility of producing the diseases in the ordinary laboratory animals for experimental investigations, although a temporary carrier state may be produced in rabbits. In all probability basic fuchsin would be a compound possessing some degree of bactericidal activity *in vivo*

for the bacilli of this group, but I have no knowledge of its being employed. Neutral acriflavin may be likewise worthy of trial, but in cases of early typhoid with positive blood cultures, I believe that one or two intravenous injections of mercurochrome in dose of 15 to 20 c.c. of a 1-per cent. solution for adults would be distinctly worth trial for attempting the disinfection of the blood and possibly of the tissues to such an extent, at least, as may reduce the duration and spread of the infection. Pond and Barrier,[68] state that the intravenous injection of this compound apparently shortened the duration of typhoid fever in 12 cases receiving one or two injections. Leitner[69] states that of 12 carriers of *Bacillus paratyphosus B* treated with one or two intravenous injections of neoarsphenamin in dose of 0.3 to 0.6 gm., 10 were apparently cleared up, but these results require confirmation.

Chemotherapy of Arthritis.—I have already discussed in Chapter VII the chemotherapy of experimental arthritis with the salicylates and related compounds. Their beneficial and curative effects in this disease cannot be satisfactorily explained at present, unless the etiological agent is an undiscovered organism much more susceptible to these compounds than are the various streptococci regarded as causing both acute and chronic forms of bacterial arthritis. Suffice to state that the administration of this group of compounds usually affords so much relief in acute rheumatic fever, at least temporarily, that the salicyls are commonly included in the list of specific drugs and various theories to explain their action have been discussed in Chapter VII. The toxicity, pharmacological action, methods of administration, etc., of the salicylates are so well known, however, that it is not required to review them here, but it may be well, at least, briefly to allude to some of the newer compounds advanced as substitutes for them in the treatment of arthritis.

Of these, phenylcinchoninic acid, better known as "cinchophen" or "atophan" and related to quinolin and salicylic acid, as well as the ethyl ester of paramethylphenylcinchoninic acid, better known as "neocinchophen," "novatophan" or "tolysin," are commanding considerable attention as indicated by the early reports of Heller,[70] Bendix,[71] Klemperer,[72] and Jokl,[73] who treated various types of arthritis with cinchophen or its derivatives. The three latter observers found neocinchophen especially beneficial in the treatment of the acute arthritis of rheumatic fever. Small doses were administered—rarely more than 3 gm. a day for from two to seven days—so that many of the patients were only partly relieved; and it was frequently necessary to change the medication to the salicylates. However, they found the drug less toxic than salicylates, and Jokl concluded that not only was this drug a good substitute for the salicylates in many cases of acute polyarthritis, but also it was to be preferred in those cases in which one hesitates to use it i.e., when rheumatic fever is complicated by nephritis, heart disease or gastro-intestinal disturbance.

The toxic effect of cinchophen was called attention to by Schroeder,[74] who reported 9 cases of his own of severe by-effects from the drug, and 8 other cases reported to him by other physicians and his patients. In his experience, the drug may produce irritation of the digestive tract, as well as constitutional symptoms of generalized itching, eruption and edema of the skin, fever, headache, etc. Hanzlik and his associates[75] found that both cinchophen and neocinchophen may produce injurious effects on the kidneys, as evidenced by albumin, casts and white blood cells in the urine, but that the effect is greater with the former drug. They also found that the symptoms of salicylism, such as nausea, vomiting, dizziness and tinnitus, may also accompany the use of these drugs.

One special advantage of cinchophen and neocinchophen over the salicylates is their effect in increasing the elimination of uric acid in the treatment of gout. The increased excretion begins almost immediately, but lasts only so long as the drug is continued; the increase is sometimes so great that uric acid is precipitated in the urine before it is voided. Clinically, however, both drugs are much less efficient in chronic gout than in acute arthritis and non-uratic joint affections. The dosage of cinchophen and neocinchophen is from 0.5 to 1 gm. (8 to 15 grins) three or four times a day; or 0.5 gm. every hour for six doses, with large amounts of water.

Plant,[76] Heubner,[77] Edens[78] and others have claimed good results in the treatment of arthritis with "maretin" and Engelen[79] and others with "melubrin" (the sodium salt of phenyl-dimethyl-pyrazolon-amido-methan-sulphuric acid), but neither has proved superior to the salicylates or cinchophen and have not commanded much attention. In my experiments solutions of "melubrin" as concentrated as 1:20 were without bactericidal effects for streptococci and staphylococci *in vitro* in exposures as long as two hours, and amounts as high as 0.3 gm. per kilo were without trypanocidal effects.

Special mention may be made of intravenous injections of mercurochrome in the treatment of infectious arthritis due to streptococci, pneumococci, staphylococci and the gonococcus. In some cases of acute arthritis with sterile blood cultures, the relief from pain and swelling is sometimes very pronounced. In gonococcus arthritis, however, the results have not been as good as in other types, although gonococcus bacteremias have been cured promptly and permanently. Young, Hill and Scott[79a] however, have recently stated that of twelve cases of gonorrheal arthritis treated with intravenous injections of mercurochrome, five were apparently cured with complete disappearance of the arthritis and initial infection. Strikingly good results were also reported in the treatment of four additional cases non gonorrheal in origin. In arthritis deformans the results have been generally negative and mercurochrome is of no value at all by intravenous injection in the treatment of primary foci of infection in the teeth, tonsils or elsewhere.

In *suppurative arthritis* of the knee caused by staphylococci, pneumococci and gonococci, Churchman[80] has irrigated with saline solution and hydrogen peroxid followed by hot 1:1000 gentian-violet for five minutes and finally with 1:10,000 gentian-violet which is left in the joint, by means of a special apparatus designed by him, and whenever a joint is opened for drainage, lavage with gentian-violet, acriflavin or a mixture of the two (1:1000 to 1:2000) may prove efficacious and especially in infections produced by the Gram-positive cocci. Mercurochrome in 1:1000 solution may be employed in the same manner. Whether or not aspiration with the injection of these disinfectants is worth while, I am unable to state; likewise, in tuberculous arthritis, the value of these procedures has not been determined, although lavage with gentian-violet by Churchman's method is worthy of consideration.

Chemotherapy of Tuberculosis.—The chemotherapy of tuberculosis is almost entirely at present in the experimental stage and I have already briefly discussed the situation in the preceding chapters in a review of the results observed with a large number of dyes, arsenicals, mercurials, copper and gold salts, the cerium earths, guaiacol and its congeners, etc., in the treatment of tuberculosis of human beings and the lower animals. Gold and copper salts have long maintained the places of primary interest in relation to the chemotherapy of tuberculous infections, but none have so far demonstrated sufficient curative activity to warrant their use in the treatment of tuberculo-

sis, although it is not to be denied that some are apparently capable of producing tuberculin-like reactions about tubercles which may prove beneficial, when frequently repeated and not excessive, by increasing the laying down of fibrous tissue and encapsulation. At this writing, sodium aurothiosulphate (sodium gold thiosulphate), better known as "Sanocrysin," is commanding attention by reason of the work of Møllgaard and his associates.[81] According to these investigators a dose of from 0.01 to 0.02 gm. per each kilogram of body weight has no harmful effect and these are the doses commonly employed along with an antiserum for preventing severe reactions believed to be caused by destroyed tubercle bacilli.

But the tuberculin-like reaction following the injection of the drug in tuberculous patients may be serious; numerous deaths have resulted, and clear-cut indications and rational dosage for the antiserum have not been established. Careful consideration of the results recorded of the "sanocrysin" treatment by the Danish clinicians reveals that it is of no value in miliary tuberculosis or in tuberculous leptomeningitis, and that in advanced and serious cases of pulmonary tuberculosis the treatment is perilous and offers "only a slight chance of recovery." Furthermore, in surgical tuberculosis, intravenous injection "has given no satisfactory results." It is apparent, therefore, that there is no justification for rushing this drug and the serum into the treatment of tuberculosis at the present time until they have been more thoroughly studied under well-controlled conditions, which investigations are now reported as being under way. My colleagues Schamberg, Harkins and Brown[81a] have recently found that gold compounds had some influence upon experimentally produced cutaneous lesions of rabbits with prolongation of life, but in no instance were cures effected.

Chaulmoogra oil, which is regarded as exerting a curative effect in leprosy, has shown no beneficial results in the treatment of experimental tuberculosis, as already reviewed in Chapter VII, but mention may be made of the report of Lukens,[82] indicating that it may be worthy of trial in the treatment of tuberculous laryngitis for the relief of pain and dysphagia. The oil is diluted to 10 to 20 per cent. with olive oil and two to three times a week from 0.5 to 1 c.c. dropped above and behind the epilogttis and finally on the cords by means of a syringe fitted with a metal eustachian catheter. Alloway and Lebensolm[83] state that daily treatments of this kind are useful in the majority of cases although they cannot entirely replace other forms of medication; Peers and Shipman,[84] however, state that, while slight temporary relief may follow and especially relief from dysphagia, caution should be exercised in selecting patients, as febrile cases may be influenced unfavorably.

In this connection mention may be also made of the effects of "sodium morrhuate," the sodium salts of the mixed fatty acids of cod liver oil, employed by Rogers and his colleagues in the treatment of tuberculosis by intravenous injection. Apparently slight febrile and focal reactions have been produced with improvement in some cases in the form of lessening of fever, cough, expectoration and numbers of tubercle bacilli, but specific tuberculocidal effects are not produced and the compound cannot be regarded as influencing tuberculosis more than cod liver oil itself.

Mention may also be made of the possibilities of chemotherapy in association with surgery in the treatment of large tuberculous pulmonary cavities as recently reported by Gekler, Lovelace, Rankin and Weigel.[85] Following the operations, a saturated solution of gentian-violet in 10-per cent. alcohol and distilled water was applied to the walls of the cavities by means of an atomizer; in some cases methylene-blue was applied after gentian-violet and they state that the latter brings away necrotic and sloughing tissue and that it will

definitely control the usual secondary infection to be found in a tuberculous cavity, and also kill off most of the tuberculous infection. Methylene-blue, following the gentian-violet, seemed to have an even more markedly deleterious influence on the tubercle bacilli, and its free use in a 4-per cent. solution did not damage the tissues or inhibit the process of repair. Furthermore, in one case, the cavity underwent rapid shrinkage and while this method of treatment is still in the experimental stage, since sufficient time has not yet elapsed to determine what will be the ultimate fate of these cavities, yet the work of Gekler and his associates is commendable and may open up a method for the chemical disinfection of tuberculous cavities of the lungs, not only by the method developed by them, but possibly also by means of direct aspiration and drainage with the application of disinfectants by means of the Jackson bronchoscope.

Arsphenamin and neoarsphenamin have also been employed in the treatment of tuberculosis and especially in tuberculosis of the skin and those cutaneous lesions of obscure origin known as "tuberculids;" I shall, however, discuss these subjects shortly in the section devoted to the chemotherapy of skin diseases.

Chemotherapy of Leprosy.—Naturally enough, in a disease of such antiquity as leprosy, numerous efforts have been made to develop or discover a specific remedy and a host of substances have been advocated, tried and discarded. At the present time chaulmoogra oil and the ethyl esters of its fatty acids are commanding most attention and, indeed, according to Warren[86] from whose excellent review of the recent literature I shall largely quote, in India and adjacent countries, chaulmoogra oil has been used both orally and externally in the treatment of leprosy since prehistoric times. However, it is so irritating to the intestinal tract that the oral dosage can seldom be pushed to the curative point in leprosy. It is a current belief among the natives of India that even if the digestive tract of a leper patient can withstand the chaulmoogra oil treatment the diseases can not always be cured, i.e., that chaulmoogra oil is not a specific in the treatment of leprosy.

The chemistry of true chaulmoogra oil was very exhaustively worked out by Power and his associates[87] a number of years ago. They found that the oil consists chiefly of the glyceryl esters of two or more new fatty acids, with a small quantity of palmitin and a phytosterol. The new acids isolated differ from any previously known fatty acids in containing a five-membered carbon ring with side chains of diminishing length as the molecular weight decreases. Also, they are unique in being optically active. They contain only one pair of doubly-linked carbon atoms; hence they absorb but two halogen atoms. The acids isolated were named respectively "chaulmoogric acid" and "hydnocarpic acid" by their discoverers and it is probable that the specific bactericidal and medicinal properties of these acids are associated in some way with their molecular constitution.

The literature on the use of chaulmoogra oil by oral administration in the treatment of leprosy is so extensive and controversial, that it is not profitable to attempt its review. The first use of the drug by subcutaneous injection appears to have been recorded by Tourtoulis[88] in 1899, who had employed it for several years with favorable results, but who did not consider the remedy a specific; Tourtoulis also used the drug intramuscularly with some success and shortly afterward Hallopeau[89] reported its use by the same method. According to Dyer, Blanc of New Orleans gave subcutaneous injections as early as 1888.

During the next decade the administration of chaulmoogra oil was still beset with difficulties. If administered by the mouth, nausea and digestive

disturbances may follow; if given subcutaneously, it is absorbed slowly since it is a heavy oil; by the intramuscular route, its administration is apt to be painful and sores are apt to develop due to slow absorption. At the present time the oral administration of the oil itself is seldom employed but preference given the subcutaneous injection of mixtures as advocated by Mercado and Heiser,[90] Heiser,[91] McCoy and Hollmann[92] and others. Rogers[93] had originally used the fatty acids of the oil by oral administration, but later employed their sodium salts (sodium gynocardate and sodium hydrocarpate) by subcutaneous and intravenous injection. Subsequently Rogers[94] was led to try the sodium salts of the mixed fatty acids of cod liver oil (sodium morrhuate) and Muir,[95] Carthew[96] and others have rendered favorable reports upon their value in leprosy. In 1918, however, Hollmann and Dean[97] advocated the subcutaneous and intramuscular injection of the ethyl esters of the fatty acids of chaulmoogra oil and this method is probably the most popular at present and particularly with the products improved upon chemically by McDonald,[98] Dean and Wrenshall.[99] Their latest work has been conducted with the esters prepared from the higher boiling fractions of the acids of chaulmoogra oil. These fractions have very high iodin and refractive index values which indicates that they belong to fatty acids which are relatively more unsaturated than the chaulmoogric series of acids. Since the hydnocarpic acid derivatives are believed to be somewhat more valuable in therapy than the corresponding chaulmoogric compounds, it is possible, as suggested by Warren, that acids of the chaulmoogric series of lower molecular weights would be still more efficient. Consequently one of the problems now open to the chemist is the synthetic production of acids of this series with shorter side chains and correspondingly lower molecular weights.

There are therefore, four main methods for the administration of chaulmoogra oil in the treatment of leprosy. In the *first* and oldest method the oil itself is given internally, but this has been largely abandoned because of gastric disturbances. Starting with a dose of 3 drops per day, it is said to be possible, however, to develop slowly a tolerance for as much as 15 drops per day. It is assumed that the emetic effects are due to local irritation, but it is a question whether almost as much nausea is not produced by any preparation of chaulmoogra oil by its central effect, irrespective of the method of administration, and oral administration is still favored by some institutions in India. It is quite possible to give by mouth weekly doses of oil, equivalent to 0.5 c.c. of the esters without producing extreme nausea or other toxic effects, but it is doubtful if oral dosage can be increased to equal that of subcutaneous injection of the esters.

In the *second* method the whole oil is injected subcutaneously, Heiser's mixture being usually employed:

Chaulmoogra oil	60 c.c. (f℥ii)
Camphorated oil	60 c.c. (f℥ii)
Resorcinal	4 gm. (gr. 60)

The mixture is sterilized at 100°C. and injected intramuscularly in ascending doses from 1 to as much as 10 c.c. at weekly intervals. Doses exceeding 5 c.c. however, are apt to produce abscesses. Reports by Hopkins,[100] Bercovits,[101] Coghill,[102] Hall,[103] Cadbury,[104] Connell[105] and numerous others, however, indicate that many apparent cures and improvements have been secured. Harper[106] has even injected the whole oil intravenously, employing a mixture of 500 c.c. of oil, 500 c.c. of ether and 1 gm. of iodin, the dose being from 10 to 20 minims at weekly intervals.

In the *third* method the soluble sodium salts of the total fatty acids of chaulmoogra oil are injected intravenously, Rogers employing a 3-per cent.

solution and injecting from 1/10 to 4/5 grain at weekly intervals. Unfortunately, however, phlebitis is sometimes produced as a result of a long series of injections.

In the *fourth* method the ethyl esters of the total fatty acids of chaulmoogra oil are injected intramuscularly or subcutaneously in doses of 0.1 to 1 c.c., Muir's formula being as follows:

Mixed ethyl esters		
Pure creosote	āā..	30 c.c. (f℥i)
Camphor		30 gm. (℥i)
Olive oil		75 c.c. (f℥ijss)

Some pain is produced, but by careful attention to technic in preparing the esters it is possible greatly to reduce local irritation by removing the irritating volative impurities. Perkins[107] employs a current of steam for two hours; in Calcutta they are removed by washing with sodium carbonate solution and in China by repeated distillations. Hollman[108] has recently described the Ball method for their preparation and Morrow, Walker and Miller[109] state that the butyl and propyl esters are less irritating than the ethyl esters. Read[110] has recently made a thorough study of the toxicity of chaulmoogra oil and its derivatives.

In addition the diet requires attention. Embrey[111] in working on the diet of lepers in the Philippines showed how marked a difference in body weight could be produced by giving attention to the vitamin and salt content of the food. Muir[112] lays particular emphasis on a diet rich in raw vegetables. The foods recommended have an alkaline ash and are rich in vitamins. Strychnin is principally of value in nerve leprosy and in nodular leprosy. The local lesions may be treated with electrocautery, *x*-rays, carbon dioxid snow or with local injections of the esters.

In conclusion, brief mention may be made of other substances in the treatment of leprosy. Thus Cawston[113] states that intravenous injections of tartar emetic are efficacious; Denney[114] has tried basic fuchsin; Takano[115] and Sugai[116] have used cyanocuprol and Hopkins[117] lists a large number of substances including tartar emetic, methylene-blue, bromids, vaccines, serums, etc. The mercurials have also been employed, the literature being reviewed by Denney and his associates[118] who have also reported that intravenous injections of mercurochrome, while not specific, aided in checking rapid retrogression of the disease. Arsphenamin and neoarsphenamin have been widely employed and the reports of de Vertenil[119], Sandes,[120] Chumokoff[121] and others as well as those of Gougerot,[122] Hasson[123] and others employing amino-arsenophenol, would tend to indicate that these arsenicals may be of some value. Not infrequently, however, syphilis is associated with leprosy and the administration of the arsenicals may give the impression of benefiting leprosy in such cases without affecting the leprous lesions themselves; on the whole nothing is to be expected in the treatment of non-syphilitic lepers with these compounds.

Chemotherapy of Anthrax.—In the chemotherapy of anthrax only those cases showing the presence of bacilli in the blood require consideration. This includes only a small fraction of cases of cutaneous anthrax, since prompt local treatment of malignant pustule usually results in an eradication of the disease. But all cases of internal anthrax are to be regarded as extremely dangerous at the outset. Blood cultures should be made on all cases irrespective of the size or location of the lesion. In one of my cases of cutaneous anthrax in whom bacteremia developed, the lesion occurred in a hair follicle of the skin of the thorax and was so small that it almost escaped detection.

In all cases of anthrax bacteremia, energetic treatment is required of the local lesion with intravenous injections of anti-anthrax serum and arsphenamin. The latter and neoarsphenamin are the only chemical compounds so far observed to exert a curative effect in this disease, although mercurochrome and gentian-violet by intravenous injection would appear to be worthy of trial. Becker,[124] Bettmann and Laubenheimer,[125] Mokrzecki,[126] Buberl,[127] Gsell,[128] Gasser[129] and numerous others have recorded cases of recovery ascribed to intravenous injections of arsphenamin or neoarsphenamin and I have already discussed in Chapter VII the results observed with these compounds in the treatment of experimental infections. Hutyra and Marck[130] also believe them worth trying in the treatment of anthrax of the lower animals.

For adults the dose of arsphenamin may be 0.6 gm. in alkaline solution injected intravenously in the usual manner; neoarsphenamin may be injected in dose of 0.9 gm. and it is usually advisable to repeat either dose in one or two days, making daily blood cultures.

Chemotherapy in Diseases of the Skin.—In a broad and general manner, diseases of the skin offer abundant opportunities for the practice of chemotherapy and especially if one accepts the local destruction of parasites, as in impetigo contagiosa, ecthyma, the trichophytoses, etc., as examples of chemotherapy in which medicaments are applied for parasiticidal effects without irritation or destruction of the body cells. I will admit that chemotherapy is not ordinarily thought of in the treatment of these purely local infections, but its principles are involved, nevertheless, although the importance of organotropism or the toxicity of chemical agents for the body cells is not nearly as great as when the medicaments are administered internally or by injection with consequently greater absorption. In some systemic or localized diseases with pronounced cutaneous lesions, as syphilis, frambesia tropica, gangosa, espundia, verruca peruana, leprosy and granuloma inguinale, chemotherapy plays a very important part and these diseases are separately considered in this or succeeding chapters.

Many of the local diseases of the skin, like impetigo contagiosa and ecthyma, are undoubtedly due to organisms, although the exact etiology has not been worked out; in these local disinfectants are of value in treatment. In others, like erythema multiforme, etiological studies strongly indicate a bacterial origin, while in others, like the herpetiform disorders and verruca, etiological studies indicate the operation of filtrable viruses, which so far have defied all efforts for identification, but against which the administration of chemical disinfectants by intravenous injection may prove of aid in treatment. In acne vulgaris, sycosis vulgaris and pyodermic infections in general, as well as in some of the eczemas and other scaly diseases, bacterial infection plays an important part and local applications of bactericidal compounds may be indicated in treatment; but these are so well known that it is hardly required or advisable to consider them in this treatise, although it may be stated in passing that the very multitude of such remedies to be found in books devoted to the diseases of the skin bears testimony to the fact that many are unsatisfactory and that the subject of cutaneous disinfection in these primary or secondary infections is one deserving of closer study and investigation from the chemotherapeutic standpoint.

Chemotherapy of Erysipelas.—A great number of substances have been applied locally in the treatment of erysipelas, a dermatitis and cellulitus caused by *Streptococcus erysipelatis*. Hot or cold local applications like saturated solutions of magnesium sulphat, lead water and laudanum, etc., may afford considerable relief even though there are no reasons for regarding them as bactericidal and capable of penetrating the skin and destroying the

streptococci in the cutaneous and subcutaneous tissues. It is possible, however, that saturated solutions of boric acid and solutions of mercurochrome may penetrate more deeply and I have used the following mixture with considerable success:

Saturated solution boric acid	400 c.c.
Alcohol (95- per cent.)	100 c.c.
Mercurochrome	0.5 gm.

This gives a 1:1000 mercurochrome in about 20-per cent. alcohol with the boric acid solution and the mixture is highly bactericidal for streptococci and staphylococci. Acriflavin may be used instead of mercurochrome in the same amount, but I believe that the latter yields somewhat better results. Either solution, hot or cold, should be frequently applied on lint or gauze and particularly at the margins where streptococcus activity is especially marked. Adams[131] has used a 5-per cent. water solution of brilliant green painted over the infected areas once or twice a day and states that beneficial results were observed in twelve cases. The discoloration can be removed in three or four days after subsidence of the infection by vigorous washing with ether soap. Of interest in this connection is the method of skin sterilization for operations practiced by Bonney and Browning[132] with a solution containing 1 per cent. of equal parts of crystal violet and brilliant green dissolved in equal parts of alcohol and water.

In severe cases, it may be necessary to administer antistreptococcus serum intravenously and Young and Birkhaug[133] have reported successful results following the intravenous injection of mercurochrome in dose of approximately 23 c.c. of a 1-per cent. solution per 100 pounds of weight. Young, Hill and Scott[133a] subsequently reported brilliant results in the treatment of seven additional cases and state that not a single failure has been brought to their attention. I have used gentian-violet by intravenous injection in dose of 30 c.c. of 0.5-per cent. solution per 100 pounds, but without success; in severe spreading infections it may be advisable, therefore, to use mercurochrome by intravenous injection and particularly if the infection cannot be brought under control by local applications and antistreptococcus serum. These measures are particularly indicated if blood cultures show that a septicemia is present. Rivanol (an acridin dye) and vuzin (a quinin compound) have also been employed by local application and intravenous injection, but I have had no experience with them in this disease.

Erysipeloid (Rosenbach) is not usually as severe as erysipelas and the etiology is doubtful, but it is very probably either a mycotic or bacterial infection in which saturated solutions of boric acid or the mixture of boric acid, alcohol and mercurophen described above, may be useful.

Chemotherapy of Cutaneous Tuberculosis and the Tuberculids.—In lupus erythematosis, Hachez[134] has reported the recovery of four cases following intravenous injections of neosilver arsphenamin in dose of 0.1 to 0.3 gm., two cases requiring eight injections before the lesions subsided. Stokes[135] states that of 20 cases with papulonecrotic tuberculids and erythema induratum treated with arsphenamin in conjunction with roentgen-ray and constitutional regimen, 17 were markedly benefited, with apparent cures in some. Many of these patients had a definite tuberculosis, the lymphadenitis in 9 cases being treated surgically without influencing the cutaneous lesions. Arsphenamin had no appreciable influence on adenitis and is believed to be contraindicated in acute febrile or rapidly progressing cases, but otherwise has shown a marked effect upon improving the general condition of the patients with an increase of weight, disappearance of rheumatic pains, etc.

Benoit,[136] Zauck and Pelbois,[137] Morin and Julia,[138] Ravaut[139] and others have likewise reported good results.

It is difficult to explain these effects but it is possible that intravenous injections, producing colloidal reactions and associated with roentgen-ray and sunlight exposures, may result in an increase of leukocytes and enzymes of the skin and blood responsible for the improvement in both the general health and cutaneous lesions. Whatever may be the mechanism, it would appear, however, that the intravenous administration of arsphenamin may prove quite helpful in the treatment of the tuberculids when combined with an improved regimen, roentgen-ray or other appropriate local measures. Needless to state, a host of substances have been employed by local application with indifferent success.

Chemotherapy of Pemphigus and Herpetic Diseases of the Skin.—As is well known the administration of arsenic is frequently beneficial in the treatment of dermatitis herpetiformis and both arsphenamin and neoarsphenamin have been administreed intravenously as substitutes for arsenious acid by ingestion, but to the best of my knowledge these compounds are of little or no benefit in the treatment of acute pemphigus. Merz,[140] Stumpke,[141] Wolfe,[142] Friboes,[143] Eschweiler[144] and others have reported favorable results, but it is possible that these occurred with bullous forms of dermatitis herpetiformis rather than with true "septic" pemphigus foliaceous and pemphigus vegetans, which are invariably fatal, although mild cases may develop remissions over a period of months. In such cases, my colleagues, Greenbaum and Schamberg, have administered mercurochrome intravenously, but, after the acute death of one case ascribed to this drug, have advised small and cautious doses like 10 or 15 c.c. of a 1-per cent. solution instead of the usual doses of 20 to 30 c.c. for an adult of average weight.

Now that herpes zoster and the various clinical types of herpes simplex are known to be caused by a virus as shown by the investigations of Goodpasture, Teague, Levaditi and others, it is to be hoped that chemotherapy may prove of aid and especially in the treatment of zoster, but at this writing no compounds have been evolved demonstrating a specific effect. With the assistance of Dr. Malcolm J. Harkins, I have produced herpetic lesions on the corneas of rats and given intravenous injections of arsphenamin, neoarsphenamin, mercurochrome, gentian-violet and acriflavin every three or four days in doses per kilogram equivalent to those ordinarily given to human beings, but the lesions did not appear to be affected or to heal more rapidly than the untreated controls. The ease with which the experimental lesions are produced, however, renders these infections readily approachable for chemotherapeutic experiments.

Chemotherapy of Mycotic Diseases of the Skin.—The chemotherapy of the mycotic infections of the skin, as the various ringworms or trichophytoses (tinea circinata, tinea tonsurans, tinea sycosis, tinea cruris, etc.), favus (achorion schoenleinii), tinea versicolor (microsporon furfur), etc., would appear to offer a particularly interesting and practical field for investigation. A host of substances have been advocated for the eradication of these infections and while some, like tincture of iodin, may be efficacious by reason of a direct mycocidal effect, others like salicylic and benzoic acids, which are the essential ingredients of the well-known Whitfield ointment, may owe their efficacy primarily to exfoliative effects. In these infections the problem would appear to be one of discovering a compound possessing a sufficiently high myocidal activity, combined with the property of penetrating the diseased skin and hair without irritation of the skin; doubtless exfoliation would greatly aid in the therapeutic effects.

Schamberg and myself[145] have conducted a number of tests with various dyes, mercurials and other substances incorporated in Sauboraud's medium to determine their relative efficiency in restraining the growth of various pathogenic molds (mycostatic tests) and also their relative efficiency in killing the molds (mycocidal tests); we observed that the two mercurial compounds (mercurophen and mercuric chlorid) exhibited the strongest myocidal effects with iodin ranking third. Among the dyes, brilliant green was most mycostatic but crystal violet proved distinctly superior as a myocidal agent. I have more recently observed that mercurochrome compares favorably with the other mercurials and because of its superior penetrating qualities I believe that it may prove worthy of use in the treatment of the various trichophytoses as a substitute for the widely used ammoniated mercury or mercuric chlorid; the following mixture has proved efficacious when employed along with the other well known local measures, the mercurochrome being finely ground before incorporation in the ointment:

Mercurochrome	gr. xxx (2 gm.)
Salicylic acid	gr. xx (1.5 gm.)
Benzoated lard	
Zinc ointment	āā ℥i (30.0 gm.)

Mercurophen may be substituted for mercurochrome and it is sometimes advantageous to employ solutions of mercurochrome, a 0.5-per cent. solution in 50-per cent. alcohol being usually well borne and penetrating deeply although objectionable for use on the exposed parts by reason of its color.

Chemotherapy of Diseases of the Eye.—In relation to chemotherapy, the diseases of the eye may be divided into two main groups, namely, (*a*) those of the deeper structures like the iris, lens, choroid, retina and optic nerve approachable only by the systemic administration of medicaments and (*b*), those of the lids, conjunctiva and cornea approachable by the direct application of chemical agents.

Insofar as the first group is concerned, the chemotherapy of congenital and acquired *syphilis* of the cornea, iris, retina, optic nerve, etc., is by all odds the most important and is separately considered, because the principles of the treatment of syphilis in general are involved and the whole subject is too important to permit of dismissal in a brief manner. In *rheumatic iritis* the internal administration of the salicylates and related compounds is usually beneficial and also belongs to this division as likewise, possibly, attempts toward the removal of uratic deposits by the administration of such drugs as cinchophen and neocinchophen. In *acute pneumococcus infections* it is possible that some beneficial effects may be secured by the internal administration of optochin (ethylhydrocuprein) hydrochlorid in doses ordinarily safe from the danger of producing toxic amblyopia, like 2 grains in capsules three or four times a day, the administration being along the lines previously discussed in the section devoted to the chemotherapy of pneumonia.

But aside from syphilis and rheumatic infections, chemotherapy has little to offer in the treatment of parasitic infections of the deeper structures. In *subacute and chronic gonococcus, streptococcus and staphylococcus iritis*, for example, it is hardly worth while to administer mercurochrome, gentian-violet, etc., because these compounds are not sufficiently penetrating or selective to reach and disinfect these local lesions, although in *acute and fulminating pyogenic infections* of these tissues, the intravenous administration of such compounds may be demanded and especially if the lesions are

associated with septicemia. Likewise in *tuberculous infections* of eye, chemotherapy has nothing to offer at present unless the gold compounds, now commanding considerable interest, should prove of value.

But when we come to consider the treatment of bacterial infections of the conjunctiva and cornea by the topical or local application of chemical disinfectants, we approach a most interesting and practical field in chemotherapy. In some mild infections the solutions applied need possess only cleansing properties, but substances possessing appreciable degrees of bactericidal activity are of distinct aid. The essential properties of a chemical agent designed for local application in infections of these structures has been discussed in Chapter III.

Owing to the brief contact between medicament and organisms, probably only a matter of seconds until the tears and inflammatory exudates dilute the chemical agent below a disinfecting threshold, frequent instillations are required and especially in the treatment of acute infections of the conjunctiva or cornea.

Needless to state, a very large number of agents have been advocated for local application in the treatment of bacterial conjunctivitis, keratitis, dacrocystitis, etc., but is not profitable to review their properties here in relation to chemotherapy. It would appear worth while, however, for the ophthalmologist to determine, if possible, the exact nature of each infection and select a chemical agent for disinfection according to the organisms present.

Ethylhydrocuprein Hydrochlorid in the Treatment of Diseases of the Eye.— Following the discovery by Morgenroth and Levy of the extremely high pneumococcidal activity of ethylhydrocuprein, Goldschmidt[146] reported some remarkable results in the treatment of pneumococcus ulcers of the cornea and as a prophylactic before operations, following local applications and instillations of solutions of this drug. Since then Gradle,[147] Puscarin,[148] Wyler,[149] Zentmayer,[150] Bedell[151] and numerous others have reported favorably upon the use of this compound, not only in the treatment of pneumococcus conjunctivitis and keratitis, but also in dacrocystitis and to some extent in other infections like gonococcus and streptococcus conjunctivitis, vernal conjunctivitis, trachoma and for the relief of photophobia in various ocular diseases.

The drug is usually employed in 1- to 2-per cent., solution applied directly to corneal ulcers or dropped into the eye. The powder itself has been rubbed into ulcers by Patton[152] with better results. Morgenroth and Ginsberg have found that 4-per cent. solutions in olive oil may be employed for corneal anesthesia and while the water 1- to 2-per cent. solutions are not usually painful, it may be necessary to first use an anesthetic when stronger solutions are employed. The drug has also been employed in the form of an ointment by Schwartzkopff (ethylhydrocuprein hydrochlorid, 0.1 gm.; atropin sulphate, 0.1 gm.; starch, 2 gm. and vaselin, 10 gm.). Gradle[153] states that the drug deteriorates rapidly in both solution and ointment and advises that a fresh solution be used every 5 to 7 days; this may account for the reports indicating that the drug apparently loses its curative effects when employed over long periods of time.

Bedell states that mixtures of ethylhydrocuprein and atropin, boric acid and zinc sulphate are compatible; I have found the same as likewise that the drug may be mixed with argyrol, silvol, neosilvol, gentian violet and acriflavin, but not with mercurophen or mercurochrome. In pneumococcus conjunctivitis and keratitis, I believe the following mixture is particularly efficacious when applied every 2 to 3 hours:

Ethylhydrocuprein hydrochlorid	1 gm.
Neosilvol	5 gm.
Water	100 c.c.

As stated by Zentmayer frequent applications (every 2 hours) are required in the treatment of acute infections and this is to be expected when it is remembered that the bactericidal effects are rapidly and sharply reduced by dilution with the tears and inflammatory exudates.

Best results have been secured in pneumococcus conjunctivitis and ulcerative keratitis; also in corneal ulcers due to trauma and as a prophylactic before operations upon the cornea. In low grade blepharitis, streptococcus conjunctivitis, gonococcus conjunctivitis, vernal conjunctivitis, trachoma, blepharospasm and photophobia, good results have been reported by some observers, although from my knowledge of its bactericidal effects I would not expect much therapeutic effect in infections other than those caused by pneumococci and related streptococci. Since dacrocystitis is frequently a pneumococcus infection, it is to be expected that the compound will prove of value in treatment after the primary causes like ethmoiditis, frontal sinusitis, hypertrophy of the turbinates, etc., have been corrected.

Mercurophen, Metaphen, Mercurochrome and the Dyes in the Treatment of Ocular Diseases.—Mercurophen and metaphen in 1:1000 solutions, have also proven of value in the treatment of pneumococcus conjunctivitis and keratitis. These compounds have a very high bactericidal action for the Gram-positive organisms including the pneumococci, streptococci, staphylococci and *B. xerosis*, and are to be recommended for use in the treatment of ocular infections due to these organisms; also for the prophylactic treatment of the conjunctiva and cornea before operations. As a general rule, 1:1000 solutions instilled every 2 hours produces no irritation, but on the contrary, usually prove soothing and slightly anesthetic. Mercurophen also possesses a high bactericidal activity for the gonococcus, *M. catarrhalis* and various other Gram-positive and negative organisms producing ocular infections. Mercurophen may be used in solution with either argyrol or neosilvol and I believe the following mixture is especially efficacious:

Mercurophen	0.01 gm.
Neosilvol	2.0 gm.
Water	100 c.c.

Mercurochrome may be employed in 1:500 solution as likewise gentian-violet or neutral acriflavin. Mixtures of neosilvol or argyrol with mercurochrome are compatible and may be employed; argyrol and neosilvol are also compatible with gentian-violet, but not with neutral acriflavin. Burnett and Gaus[154] and Clapp and Martin[155] have found instillations of mercurochrome in 1-per cent. solution efficacious in the treatment of gonococcus conjunctivitis of adults and the new-born.

Mercurophen and boric acid are incompatible, but mercurochrome and boric acid may be used together in the same solution and it is sometimes advisable to add 0.2 gm. to each 100 c.c. of boric acid solution for general use in the treatment of bacterial infections of the conjunctiva and cornea and for preparatory cleansing for operations.

Chemotherapy in Diseases of the Ear, Nose and Throat.—In the chemotherapy of diseases of the ear, nose, throat and accessory sinuses, most interest is commanded by the therapeutic efficiency of local applications of various chemical agents in the treatment of acute and chronic bacterial infections. In the treatment of syphilis of these organs, the general principles are the same as in the treatment of syphilis of other organs and the subject is of such

importance as to demand separate and detailed consideration. I have already discussed in this chapter the treatment of suppurative meningitis secondary to otitic infections and likewise the use of chaulmoogra oil in tuberculous laryngitis; also, in Chapter III, the principles of chemotherapy by the local or topical application of chemical disinfectants, which is a subject of particular interest in the chemotherapy of bacterial infections of these organs. Plaut-Vincent's angina is discussed in Chapter XXXIII.

Needless to state, a very large number of chemical agents and mixtures have been advocated for local application in infections of these parts, but it is not profitable to review them here in relation to the general subject of chemotherapy. Doubtless, most of them would be far more satisfactory if they could be applied more frequently and more thoroughly; undoubtedly the difficulty or practical impossibility of applying chemical disinfectants to the infected parts with sufficient frequency is largely responsible for the present unsatisfactory state of the treatment of otitis media and the infections of the various nasal accessory sinuses. Largely for this reason the treatment of ocular infections is on a more satisfactory basis; not because equally good disinfectants may not be used in otitic and nasal infections, but simply because they cannot be ordinarily applied in the latter with sufficient frequency. The matter of defective and insufficient drainage is also of great importance and especially in infections of the ear and nasal accessory sinuses. Therefore, it may be stated that in a broad and general manner the unsatisfactory results of local applications of disinfectants in bacterial infections of the ear, throat, nose and accessory sinuses is due not so much to a lack of suitable and efficient chemical disinfectants, as to the fact that they cannot be, or are not, applied with sufficient frequency and that drainage is frequently insufficient. Furthermore, the disinfectant frequently fails to reach the infected tissues altogether; possibly gaseous disinfectants, like chlorin, may improve results by reason of penetrating to parts not otherwise reachable under ordinary conditions. Experience indicates, however, that chlorin is not very beneficial in the presence of suppuration and collections of pus and has little to offer in the treatment of infections of the various accessory sinuses.

Chemotherapy of Nasal and Sinus Infections.—In my experience the majority of infections of the antrum of Highmore, ethmoid, sphenoid and frontal sinuses are due to pneumococci; in chronic infections, other organisms like *B. pyocyaneus*, staphylococci and diphtheroid bacilli may also be found in smears and cultures, but a pneumococcus is apt to be the primary etiological factor. Defective drainage and the difficulty or impossibility of applying disinfectants at frequent intervals would appear to be largely responsible for the unsatisfactory state of treatment by local applications, but insofar as the choice of disinfectant is concerned, I wish to call attention to ethylhydrocuprein hydrochlorid. This substance possesses an extremely high pneumococcidal activity in the presence of pus and can be counted upon to kill the organisms if brought in contact with them. Unfortunately, however, it does not penetrate deeply but may be combined with various other disinfectants to advantage in dose of 0.01 gm. dissolved in 100 c.c. of a saturated solution of boric acid, 5-per cent. neosilvol, 1:1000 gentian-violet or 1:1000 neutral acriflavin. Unfortunately, mixtures with mercurophen and mercurochrome are not compatible.

Mercurophen in 1:1000 solution is also highly bactericidal for pneumococci, streptococci, staphylococci, *B. friedländer*, diphtheroid bacilli and *B. pyocyaneus* and has proven useful in the treatment of these sinus infections; 1:1000 solutions of mercurochrome, metaphen, acriflavin or gentian-

violet in water are likewise useful and well borne, and both mercurochrome and the two dyes may be prepared to advantage with a saturated solution of boric acid instead of water. Freshly prepared Dakin's solution containing 0.4- to 0.5-per cent. of chlorin is especially useful when the pus is very thick and it is sometimes advantageous to increase its bactericidal effect by adding 0.01 gm. of mercurochrome to each 100 c.c. Chloramin-T in 2-per cent. solution is somewhat more stable and its bactericidal action more prolonged; mercurochrome may be added if a higher bactericidal effect is desired. It is sometimes efficacious to cleanse the infected parts with Dakin's solution or chloramin-T and to follow with applications of ethylhydrocuprein, mercurophen or mercurochrome in dose of 0.01 gm. dissolved in 100 c.c. of 5- to 10-per cent. neosilvol. If gentian-violet or neutral acriflavin are used alone the solutions may be stronger, as 1:200 to 1:300; gentian-violet may be used with neosilvol, but acriflavin is incompatible.

In rhinitis and ozena frequent instillations of 2- to 5-per cent. solutions of dichloramin-T in olive, almond or paraffin oil may be employed; if argyrol or neosilvol is used, it is sometimes advantageous to render them more bactericidal by adding 0.01 gm. of either mercurochrome or mercurophen to each 100 c.c.

Chemotherapy of Diseases of the Throat and Ear.—The treatment of *Plaut-Vincent's angina* with arsphenamin and congeners is considered in Chapter XXXIII, and the subject need not be considered here with much detail. In severe infections, however, and those proving resistant to treatment with arsphenamin or one of its substitutes, additional measures may be required. For such cases 1:200 solutions of gentian-violet or neutral acriflavin, 1:500 mercurophen, 1:100 mercurochrome or 7 per cent. chromic acid may be used locally by direct application after preliminary cleansing with hydrogen peroxid; due care should be exercised against swallowing these compounds, although there is but little or no danger from poisoning. Frequent applications are required, every two or three hours, until the disease is brought under control and in severe cases, preliminary cleansing with saline solution, diluted hydrogen peroxid or Dakin's solution may be required for removing the exudates. Of interest in this connection is the report by Sutton[156] of a case of Vincent's angina developing in a syphilitic under treatment with intramuscular injections of sulpharsphenamin and indicating that better results are to be obtained by local applications of arsphenamin or one of its substitutes.

In staphylococcus, streptococcus and pneumococcus *tonsillitis* and *pharyngitis*, mercurophen or metaphen applied either in the form of a gargle, atomizer or direct application affords quick relief. For the atomizer or for gargling these compounds may be employed by dissolving 0.2 gm. in 100 c.c. of *liquor antisepticus* and diluting one part with four or five parts of water; there is no danger from swallowing small amounts, although this should be avoided. For local application by the physician I believe a mixture prepared by dissolving 0.1 gm. mercurophen in 50 c.c. of water and adding 50 c.c. of glycerin is better than a water solution. If it is desired to use boric acid, a mixture prepared by dissolving 0.1 gm. of mercurochrome and 1 gram of boric acid in 50 c.c. of water and adding 50 c.c. of glycerin, may be recommended; unfortunately mercurophen is not compatible with boric acid.

Neutral acriflavin in 1:250 solution is also useful and the compound may be employed in the form of troches. Churchman and Herz[157] and Saurman[158] have employed solutions of gentian-violet for the treatment of *diphtheria bacillus carriers*, a 2 per cent. solution being thoroughly applied by spraying four times a day after preliminary cleansing with saline. The

patient and family should be informed that the dye, though staining everything with which it comes in contact, will not leave any permanent discoloration of the skin, mucous membrane or teeth.

I have had no experience with gentian-violet in the treatment of diphtheria carriers, but, in coöperation with Woody and Yagle,[159] have tried 1:250 solutions of brilliant green applied to the throats, noses and ears of carriers three times a day. Browning[160] states that solutions of this compound sprayed over the fauces, tonsils and pharynx three times daily for a week, succeeded in eliminating diphtheria bacilli from the throats of carriers, but in our experience the disappearance of the bacilli was only temporary. Brilliant green and gentian-violet, however, are highly bactericidal for bacilli of the diphtheria group and in 1:200 solution may be worthy of use in the treatment of these and staphylococcus infections of the ear, nose and throat. Gray and Meyer[161] have recently observed that applications of 0.5- to 2-per cent. solutions of mercurochrome may materially reduce the duration of diphtheria bacilli in the nose and throat.

Abraham[162] has recently recommended gentian-violet very highly for the treatment of streptococcus infections of the pharynx, tonsils and larynx; also for the treatment of Vincent's angina and infections of the external and middle ear and mastoid. It would appear, therefore, that gentian-violet, acriflavin, mercurophen, mercurochrome and ethylhydrocuprein hydrochlorid are all worthy additions to the usual chemical disinfectants for the treatment of bacterial infections of ear, nose, throat and accessory sinuses. Callison[163] has reported good results from local applications of 1-per cent. mercurochrome in the treatment of otitis media and other otitic suppurations. Special mention may be made of the beneficial effects of mercurochrome by intravenous injection in severe *mastoiditis* and *lateral sinus* infections with septicemia or threatened infection of the blood and meninges. Injections are usually possible even in young children and single doses have been reported as bringing about prompt and marked improvement.

Chemotherapy of Diseases of the Digestive Organs and Peritoneum. The chemotherapy of bacterial diseases of the digestive organs is a subject of commanding interest presenting numerous problems of considerable academic and practical importance. The treatment of syphilis of these organs is in itself a broad subject and is considered separately in Chapter XLXV, with special reference to syphilis of the liver; the chemotherapy of amebic dysentery and other protozoal and metazoal infestments is considered in Chapter XXXIII.

Chemotherapy of Diseases of the Mouth.—In *thrush* or other mycotic infections applications of 1:200 solutions of neutral acriflavin have been recommended by Maier[164] and others; gentian-violet also yields good results in the same strength. For severe and intractable cases, 1:500 mercurochrome or 1:1000 mercurophen may be applied locally, although proper attention to dietary errors and hygiene along with cleansing with simple boric acid solution, are generally sufficient.

In my experience, local applications to aphthous *ulcers* of the tongue and cheek showing no organisms in smears or cultures other than the common mouth bacteria, are of little or no use, since the underlying causes is in the lower tract; excessive smoking, etc., require correction. Needless to state, syphilitic ulcers and mucous patches as well as ulcerous epitheliomata are not benefited by local applications, the former usually disappearing rapidly under appropriate antisyphilitic medication.

Chemotherapy has nothing to offer in the treatment of *apical abscesses of the teeth;* apparently extraction and drainage are required for their eradication. Even repeated intravenous injections of mercurochrome, gentian-violet and acriflavin are without appreciable effect upon these areas of local infection, largely because the compounds are lacking in penetrability and selective affinity.

I have considered the treatment of spirochetic *gingivitis* with local applications of arsphenamin and its substitutes in Chapter XXXIII. The treatment of these infections is practically a speciality in dentistry worthy of support on the part of both the medical and dental professions, since most cases require appropriate surgical treatment in addition to the judicious application of disinfectants. Needless to state, a host of substances are in common use and particularly tincture of iodin. The dyes are unsuitable because of staining, but local applications and injection of the pyorrheal pockets with 5-per cent. dichloramin-T in oil, 1- to 3-per cent. solutions of mercurochrome as reported by Darnall,[165] 1:500 mercurophen, etc., in addition to a similar employment of 2-per cent. solutions of arsphenamin, are all measures of recognized value when properly employed. For the treatment of very mild and superficial infections and for prophylactic use after recovery, I believe that my tooth paste of arsphenamin and mercurophen, described in Chapter XXXIII, has proved of value when used once or twice daily as a substitute for ordinary dentrifices.

Chemotherapy of Diseases of the Liver and Gall-tract.—Unfortunately, the possibilities of treating cholangitis, cholecystitis and hepatitis of bacterial origin by the administration of drugs eliminated in the bile, has not commanded as much attention as demanded by the frequency and seriousness of these diseases. Just as it is possible to influence pyelitis and other infections of the kidney and genito-urinary organs by the administration of mercurochrome and hexamethylamin, by reason of their elimination by the kidneys and raising the bactericidal activity of the urine, so it may be that compounds will be produced capable of raising the bactericidal activity of the bile to a therapeutic threshold for the chemotherapy of infections of the gall-tract with streptococci, staphylococci, *B. coli*, *B. typhosus* and other organisms. Mercury compounds would appear to be of promise, because they are usually eliminated in an active bactericidal state. But the doses of ordinary mercurials are so small, in order to avoid toxic reactions, that it is doubtful if sufficient may be given appreciably to raise the bactericidal activity of the bile. Mercurochrome, however, is of greater promise in this direction, because of the possibility of giving larger doses by reason of its toxicity being at least ten to twelve times less than mercuric chlorid and similar compounds. Hill and Scott[166] have recently studied this subject in experiments employing rabbits and state that mercurochrome is excreted by the liver and renders the bile bactericidal for typhoid and cholera bacilli. In their opinion mercurochrome has cured rabbits that had been made gall-bladder carriers of typhoid bacilli and they believe this drug meets the requirements of a biliary antiseptic, since its toxicity is low enough to allow its safe clinical use intravenously or by mouth. It is suggested that the trial of mercurochrome clinically in both latent and acute gallbladder infections is not only justified but also clearly indicated from these experimental findings.

With the assistance of Miss Anna Rule, I have administered mercuric chlorid, mercurochrome, hexy-resorcinol and arsphenamin to rabbits by ingestion and intravenous injection and have studied the bactericidal activity of the bile for streptococci, staphylococci and *B. coli* but without constant or striking results. Our plan was to administer the

drugs to rabbits by intravenous injection or by stomach tube and two hours after the last dose to etherize each animal and aspirate the bile under sterile conditions. Each specimen was divided into three parts and seeded with broth cultures of a hemolytic streptococcus, *staphylococcus pyogenes aureus* and *B. coli;* the mixtures were kept at 37°C. and subcultured at the end of 10 and 60 minutes and again at the end of 24 hours. The drugs and doses were as follows:

(a) Mercurochrome, intravenously, single doses of 0.005 to 0.025 gm. per kilo.

(b) Mercurochrome, by stomach, three doses per day for three days of 0.005 gm. per kilo.

(c) Hexy-resorcinol, by stomach, three doses per day for three days of 0.05 gm. per kilo.

(d) Mercuric chlorid, by stomach, three doses per day for three days of 0.001 gm. per kilo.

(e) Arsphenamin, intravenously, single doses of 0.01 to 0.05 gm. per kilo.

Numerous untreated controls were included and the sterile bile was found frequently to be bactericidal for the streptotoccus even in 10 minutes. The bile of mercurochrome treated animals was occasionally more bactericidal for all three organisms than the bile of normal control rabbits and especially in exposures of one and twenty-four hours but hexy-resorcinol, mercuric chlorid and arsphenamin yielded entirely negative results. I am mentioning our results here, however, to indicate that by experimental methods it is possible to study this interesting problem and that chemotherapeutic research along these lines may succeed in evolving drugs eliminated in sufficient amounts and in a sufficiently active state, to raise the bactericidal titer of the bile to a therapeutic threshold for the treatment of bacterial infections of the gall-tract and liver, including gall-bladder carriers of typhoid bacilli. Compounds largely eliminated in the bile are of most interest and it is possible that cholecystography may be of assistance in work of this kind along the lines developed by Graham, Cole and Copher[167] with tetrabromphenolphthalein.

Chemotherapy of Bacterial Diseases of the Gastro-inestinal Tract; Intestinal Antisepsis.—Naturally, the question of chemical disinfection of the gastro-intestinal tract, and particularly of the colon, has commanded a great deal of interest and attention, not only in reference to the treatment of specific bacterial infections like typhoid fever, cholera and bacillary dysentery, but likewise in relation to other forms of ileo-colitis of bacterial origin and to the possibility of preventing the production of bacterial toxic products commonly regarded as responsible for some forms of "intestinal toxemia," arterio-sclerosis and hypertension, and possibly even of focal infection of the joints and other organs.

The subject may be divided, therefore, into two main divisions, namely, (*a*) chemical disinfection of such specific organisms as typhoid, paratyphoid, cholera and dysentery bacilli and (*b*), the reduction or eradication by chemical disinfection of organisms usually present, but increased for some reason to pathogenic proportions, with special reference to bacteria of the *B. coli* group. The difficulties are many and diverse. The stomach may be readily enough washed out with disinfectant solutions and the oral administration of such disinfectants as mercurochrome, mercurophen, gentian-violet, acriflavin, etc., must surely exert some bactericidal effects, but whether or not all organisms protected by the folds of mucosa are removed is open to question, although the subject of gastric disinfection is not one of much therapeutic importance except in relation to the passage of tubes into the duodenum for the purpose of securing bile and duodenal contents for bacteriological examination. Likewise, partial disinfection of the colon may be accomplished by frequent colonic irrigations with bactericidal solutions, but to be effective special methods and skill are usually required and re-infection soon follows.

But disinfection of the small and large intestines by the oral or intravenous administration of drugs has not been accomplished, although out of the host of compounds advocated for intestinal disinfection possibly trimethylomethyloxyphenol ("trimethol") and dimethylomethoxyphenol ("dimethol") are deserving of consideration, in view of the reports of

Roberts[168] and others of their beneficial influence in colitis. But while the proteolytic and aciduric groups may be influenced by diet and probably reduced by disinfectants or the administration of cultures of *B. acidophilus*, complete sterilization of the intestines is probably impossible as indicated by the work of the Dragstedts and Nisbet[169] with isolated loops of dog intestine treated with ether, alcohol (70-per cent.) lysol (2-per cent.), silver nitrate (5-per cent.) saturated solution of zinc chlorid, phenol (5-per cent.), saturated solution of salol, thymol (10-per cent.) camphor, menthol, Dakin's solution, betanaphthol, saturated solution of quinin sulphate and mercuric chlorid, all of which failed to effect complete disinfection. Despite the fact that this procedure offers a far more direct opportunity for bactericidal effects to manifest themselves than the usual oral administration of disinfectants could possibly permit, sterilization was in no case secured. In spite of the favorable conditions of these experiments, it was not possible to prevent the intestinal toxemia associated with stasis. At present, therefore, no experimental justification can be cited for the clinical use of antiseptics intended to produce intestinal antisepsis by direct action on the microorganisms that inhabit the gastro-enteric tract, insofar as complete disinfection of typhoid, dysentery and other pathogenic organisms are concerned, although it may be possible to reduce the usual flora.

But it is possible that future researches will produce chemical agents capable of partially disinfecting the gastro-intestinal tract of the usual proteolytic and aciduric floras and totally of such foreign organisms as the dysentery bacilli, when administered by oral administration or duodenal lavage. The latter method of administration is of course more promising of success, because of the large amount of fluid that may be introduced, but it is sharply limited in its clinical applications. It may be possible, however, to secure some degree of local disinfection of the intestinal tract through the action of drugs eliminated in the bile and by the intestinal mucosa in an active bactericidal state. The full- or half-complex compounds of mercury or other heavy metals, for example, may be utilized for such purposes on the basis of elimination practically unchanged before ionization or dissociation has occurred following intravenous or intramuscular injection. But unless the compound were endowed with an extremely high bactericidal activity along with an extremely low toxicity for the body-cells, it would be impossible to administer sufficient amounts capable of materially influencing the enormous bacterial flora of the intestinal contents. Unfortunately, it would appear at present that compounds of mercury and other heavy metals possessing a low organotropism or toxicity for the body-cells, by reason of slow ionization or dissociation of the metallic ions, are likewise of reduced parasitropic activity; in other words, both toxicity for the host and toxicity for the parasites is reduced in full- or half-complex compounds of slow dissociation, although a way and means may be found for evolving compounds of low toxicity for the body-cells while possessing a sufficiently high bactericidal activity to be of therapeutic efficiency. Mercurochrome, for example, approaches this ideal more closely than other mercurial compounds and may be eliminated in the urine in bactericidal amounts following the administration of non-toxic doses.

With the assistance of Miss Rule, I have studied the effects of this compound, as well as of hexy-resorcinol and mercuric chlorid (as a control), on the gastro-intestinal flora of rabbits. Our plan was to administer the compounds according to body weight during a period of fasting in the same amounts and by the same routes listed on page 180 and then two hours later open the abdomen under ether anesthesia for bacteriological examinations. For these purposes the stomach was tied off and 10 c.c. of sterile saline injected, mixed about, withdrawn and plated. Similar tests were conducted by tying off 5 cm. of

duodenum, ileum and ascending colon and securing material in the same manner. Control animals kept under identical conditions were cultured at the same time and the results compared with those observed among the treated animals. The results have indicated that mercurochrome by oral and intravenous injection may slightly reduce the numbers of bacteria in the small, but not in the large, intestines of rabbits. The results may not have a direct clinical application at present, but I am mentioning them here in this brief manner to indicate the possibilities of chemotherapeutic research along these lines.

It is not at all unreasonable to hope for the synthesis of compounds capable of influencing bacilli of the typhoid-dysentery group either by enteral or parenteral administration; in bacillary dysentery we have the additional means of reaching the infected tissues by colonic irrigation. Indeed, I am rather inclined to believe that the oral administration of mercurochrome and possibly of trimethol and dimethol, may be worthy of attention from the standpoint of reducing the intestinal flora to a point of therapeutic efficiency and in the treatment of bacillary dysentery and other forms of colitis; possibly much may be accomplished by *frequent* irrigations with a cleansing solution followed by the injection of such compounds as mercurochrome (:3000), mercurophen (1:5000), metaphen (1:5000), acriflavin (1:3000), gentian-violet (1:3000) or acid fuchsin (1:3000). Even though solutions of these strengths were used in amounts of 2000 c.c. and retained for ten or fifteen minutes, which would be desirable, toxic effects need not be feared from either local irritation or absorption, since the larger part is returned, although it would be advisable to follow the mercurials with a second cleansing irrigation. The mercurials in these strengths are bactericidal for both Gram-positive and Gram-negative organisms, but gentian-violet would be effective principally against the Gram-positive aërobes and anaërobes, while acriflavin and acid fuchsin would be effective especially against the Gram negative bacilli.

Chemotherapy of Peritonitis.—Under this heading, I refer to the possibilities of chemical disinfection of the peritoneum following the rupture of typhoid ulcers, gangrenous appendices, shot and stab wounds of the stomach and intestines, etc., that is, the acute generalized contaminations and infections. In operations upon cases of this kind, it may be advisable to wring out the towels and gauze brought in contact with the intestines and parietal peritoneum with weak solutions of disinfectant instead of plain saline solution, like a hot 1:5000 solution of mercurochrome in saline solution; likewise, to employ a solution of this kind for flushing the peritoneal cavity at the close of the operation. Equal parts of 1:2000 solutions of gentian-violet and neutral acriflavin may likewise prove serviceable. Under these conditions, solutions of these strengths are highly bactericidal for both Gram-positive and negative bacilli and cocci including the anaërobes; furthermore, they act quickly, which is an essential requirement. Judging from experiments with rabbits, they are not irritating or antiphagocytic and even should amounts up to 1000 c.c. be retained in human adults, which is advisable, they are not to be feared from the standpoint of toxicity. For example, 1000 c.c. of 1:5000 mercurochrome contains 0.2 gm. of compound and for an adult of approximately 135 pounds of weight this amount would be about 0.003 gm. per kilogram, which is less than the full dose by intravenous injection (0.005 gm. per kilogram). Furthermore, the absorption of this amount may aid in preventing septicemia and in any event, a careful watch for this complication should be maintained with the aid of blood cultures, and mercurochrome or gentian-violet administered intravenously, as previously described, if positive cultures are observed. The dyes are even less toxic. It may not be possible to sterilize the peritoneal cavity completely in this manner, but the infection is very probably reduced numerically and this may be sufficient for insuring a

successful outcome. Young, Hill and Scott[169a] state that intraveous injections of mercurochrome may be of some aid in the treatment of postoperative peritonitis.

I do not presume to advise surgeons upon the important topic of when and when not to irrigate and I realize that in localized peritonitis with complete or partial walling off irrigation may be harmful and is to be avoided; but when irrigation is possible, I believe that something more may be accomplished by using solutions of this kind than is secured by using plain sterile water or saline solution. Unfortunately, frequent irrigations of the peritoneal cavity every two or three hours, by means of the Carrel tubes or modifications of these, may not be possible because of shock, pain, excessive peristalsis, etc., but, in the presence of suppurative peritonitis or extensive soiling of the peritoneal cavity with intestinal contents, I believe that attempts of this kind with hot Dakin's solution, boric acid solution or plain saline, are advisable and once or twice during the day a 1:2000 solution of mercurochrome may be substituted for them. It is possible that a method of this kind may likewise stimulate peristalsis and aid in preventing the much dreaded post-operative ileus, although excessive peristalsis would be objectionable from the standpoint of hindering the localization of infection. In pelvic peritonitis of women irrigation may be more successful and especially if vaginal drainage is provided.

Chemotherapy of Diseases of the Genito-urinary Organs.—Largely owing to the investigations of Young, White and Swartz[170] considerable advances have been made during the past five years in the chemotherapy of bacterial infections of the kidney, urinary bladder and genital organs with mercurochrome, employed first by local application but more recently by intravenous injection as well.

Needless to state, a great many chemical agents have been advocated and employed for the treatment of urethritis, cystitis and pyelitis. For local application, Davis and White[171] and Leonard[172] have enumerated seven qualifications for the ideal urinary antiseptic, believing that mercurochrome and hexy-resorcinol may meet these conditions, as follows:

(1) It should be chemically stable.
(2) It should be non-toxic.
(3) It should be nonirritating to the urinary tract.
(4) It should exert an antiseptic action in high dilution in urine of any reaction.
(5) It should be eliminated in high percentage by the kidney.
(6) It should be eliminated in the urine in sufficient concentration to exert a local antiseptic action and at a rate by which continuous antiseptic action may be attained.
(7) It should be administrable by mouth.

I have already discussed the properties of mercurochrome in Chapter VII but it may be well here to briefly refer to hexy-resorcinol, although the compound has not yet been employed long enough to permit definite statements regarding its chemotherapeutic and practical value as a urinary antiseptic. In 1913 Johnson[173] synthesized the lower homologues of a series of alkyl resorcinols and Rettger, in testing their bactericidal properties, found a rapid increase in the phenol coefficient as the series was ascended. Leonard[114] later discovered that alkylation of resorcinol decreases its toxicity and at the same time increases its bactericidal power in direct proportion to the sum of the atomic weights of the atoms in the side chain. The greatest bactericidal effect is reached in normal hexylresorcinol, which has a phenol coefficient of 46 to 52. The alkyl resorcinols are said to be among the most powerful organic germicides ever described as possessing anything like its degree of nontoxicity to animals and to man.

The clinical cases, although few in number (nine), gave evidence that the drug may be of promise as a urinary disinfectant on account of its strong bactericidal action in either acid or alkaline urine, the rapid and continuous excretion over a considerable period of time following oral administration, and its low toxicity.

Chemotherapy of Infections of the Kidneys.—In the treatment of pyelitis and pyelonephritis due to *B. coli*, *B. lactis äerogenes*, *B. typhosus*, streptococci, staphylococci and other organisms, about the only drugs meriting consideration from the standpoint of chemotherapy are hexamethylamin (urotropin), mercurochrome and hexy-resorcinol. Hexamethylamin unfortunately has not fulfilled original hopes and expectations, because formaldehyd is liberated only in acid urine and, as shown by Stohl and Denning,[175] the concentration must reach at least 1:20,000 to 1:30,000 to be bactericidal. This requires the administration of large doses, but as recently reported by Helmholtz and Milliken,[176] the urine may be kept sufficiently acid (*pH* 5.8 to 7) by the oral administration of either calcium chlorid or ammonium chlorid, while hexamethylamin is given in doses of 1 to 1.5 gm. (15 to 20 grains) four times a day along with large amounts of water. While they never found the formaldehyd content of the urine to reach 1:20,000, yet good results were secured in the treatment of pyelitis of children by this method of administration.

Mercurochrome in 1-per cent. solution has been employed for pelvic lavage in adults with apparently good results, although the staining may interfere with functional kidney tests. According to Young and his associates, it has about the same bactericidal activity as 1-per cent. silver nitrate with the added advantage of being less painful. Of course, pyelitis due to focal infection from the teeth or tonsils may not be cured by lavage alone, but in severe infections the drug may be administered by intravenous injection in dose of about 20 c.c. of 1-per cent. solution per 100 pounds of weight with beneficial results. Great care, however, must be exercised if the kidneys are eliminating poorly with albumin and casts in the urine; under such circumstances the intravenous dose should not exceed 10 c.c. per 100 pounds and in some cases should not be administered by this route at all.

As shown by Young, Scott and Hill,[177] the drug may be given by mouth with little or no gastro-intestinal disturbances, in dose of 0.2 or 0.3 gm. two or three times a day in capsules, as the urine shows colorimetrically a dilution of between 1:15,000 to 1:40,000 which is sufficient for rendering it bacteriostatic. An added advantage may be a reduction in the bacterial flora of the intestine, which may eliminate a urinary infection. But according to Young and Hill,[178] intravenous injections have been particularly efficacious in the treatment of bilateral chronic pyelitis caused by *B. coli* and *B. lactis aërogenes*, in pyonephosis secondary to carcinoma of the bladder, in pyelitis of children, and in retroperitoneal and perinephritic abscesses.

Young and Hill have also reported apparent cures following intravenous injections of gentian-violet and especially in staphylococcus infections, but pelvic lavage with this dye has not been used sufficiently to express an opinion of its value. Davis and Beck[179] found proflavin and acriflavin by oral and intravenous administration apparently efficacious in the treatment of urinary infections due to staphylococci and *B. coli* and especially in the presence of alkaline urine, the dose by mouth being about 0.05 gm. for adults, but Helmholtz and Milliken used this dye by oral administration in dose of 0.01 to 0.025 gm. ($\frac{1}{6}$ to $\frac{1}{4}$ grain) every six to eight hours without benefit in the treatment of three cases of pyelitis of children. Davis[180] has studied the dyes with great care and industry from the standpoint of urinary antisepsis

and of 204 tested found only 28 sufficiently antiseptic to command attention and of these only 13 which were eliminated in bactericidal amounts following the intravenous injection of non-toxic amounts (approximately 0.020 gm. per kilo of weight). Davis and White[181] have also studied the compounds related to phenolsulphonphthalein, but found none possessing antiseptic activity in the urine; chlor-mercury-fluorescein compounds, however, were found to be eliminated by dogs in antiseptic amounts. It is of interest to note in passing that Davis[182] also found that the normal urine of dogs was antiseptic for the bacilli of the colon-typhoid group, but much less so far staphylococci; these observations indicate, therefore, that due care must be exercised in technic and interpretation of results when these animals are employed for experiments on urinary antisepsis.

Leonard has found the oral administration of 0.5 to 1 gm. of hexy-resorcinol in olive oil in capsules three times a day, apparently efficacious in the treatment of a few cases of urinary infections and it would appear that this drug, along with mercurochrome by pelvic lavage, oral or intravenous administration, are the most encouraging and valuable drugs available at present for the chemotherapy of kidney infections. To the best of my knowledge they have not been reported upon in the treatment of tuberculosis of the kidneys and are not likely to be of value in this infection, but in streptococcus, staphylococcus or *B. coli* pyonephritis, it would appear that mercurochrome and hexy-resorcinol are worthy of trial, although great care must be exercised in the intravenous administration of the former. I may state however, that a brief experience with hexy-resorcinol has not so far yielded noteworthy results in kidney and bladder infections; furthermore, some patients cannot take the drug because of nausea and other effects. Hexy-resorcinol is still in the experimental stage at this writing and it is too early to express any definite opinion of its practical value.

Chemotherapy of Cystitis.—In cystitis caused by *B. coli* or a bacillus of this group, streptococci, staphylococci, etc., good results have been secured by the instillation of mercurochrome and especially in *B. coli* infections. It is well to first cleanse the bladder with sterile saline solution and then to inject a freshly prepared 0.5-per cent. solution. Two instillations per day may be given to bring the infection under control as soon as possible and the strength of the solution may be raised to 1-per cent., if well borne, as it usually is, although some patients can scarcely tolerate the 0.5-per cent. solution. The frequency of instillations should be guided by the symptoms and bacteriological examinations of the urine.

Mercurophen in 1:4000 to 1:10,000 solution has likewise proven efficacious by instillation, according to the same plan and is especially useful in the treatment of cases caused by the Gram-positive cocci (staphylococci, streptococci and pneumococci) in which mercurochrome is more likely to fail.

The action of both drugs locally may be enhanced by the oral administration of mercurochrome in dose of 0.2 gm. (3 grains) or hexy-resorcinol in dose of 0.5 gm. (8 grains) three times a day, although the urine must be carefully watched for albumin and casts. Urotropin may likewise prove of some value, provided the urine is kept acid and large doses (1 to 2 gms.) are given three times a day along with large amounts of water.

Chemotherapy of Urethritis and Urethro-prostatitis.—As is well known, a very large number of chemical agents have been advocated for local injection in the treatment of gonococcus infection of the urethra and adnexa of both sexes. The difficulties of chemical disinfection are very great and principally by reason of the fact that efficient bactericides may prove too irritating or fail to reach the gonococci and organisms of secondary infection (staphylo-

cocci, diphtheroid bacilli, *B. coli*, etc.) deeply entrenched in the glands of the urethra and prostate gland. The colloidal and colloidal protein silver preparations and potassium permanganate have long proven favorite disinfectants, Paul,[183] Bruck[184] and others having studied their bactericidal properties very extensively, although, as recently shown by Sollman and Pilcher,[185] the bactericidal activity of these compounds is low and dependent upon the amount of free silver ions immediately available which varies from 7.5 per cent. in the case of protargol to 1.5 per cent. for collargol, 0.4 per cent. for argyrol and to 0.2 per cent. for silvol; they state that these colloidal preparations do not furnish reserve depots for the dissociation of the active ions as commonly believed, and that their value is chiefly soothing and demulcent in addition to what bactericidal action results from the silver ions immediately available. Ballenger and Felder,[186] while observing that a colloidal silver chlorid was highly bactericidal *in vitro*, found that it was of no special value in the treatment of well-established cases of gonorrhea. McDonagh[187] has advocated local injections of colloidal manganese and colloidal palladium; Gladstone[188] has also employed manganese and intramine, and so on through an almost interminable list.

Acriflavin has been widely used for the treatment of gonorrhea in 0.5-per cent. solution and is apparently helpful in many cases. Thus Davis and Harrell[189] found that 1:300,000 solutions were bactericidal for the gonococcus *in vitro* and that local injections were efficacious in the treatment of gonorrhea and capable of penetrating the mucosa of the urethra and bladder; 3 c.c. of 1:1000 solution, 15 to 30 c.c. in posterior cases, were injected twice daily with only slight irritation. The average duration of the disease was believed to have been shortened, but some cases were not benefited. Watson,[190] Armstrong[191] and others have also used the compound by local injection and Wood[192] and Jacob and Verasingan[193] have advised intravenous injections of 150 to 300 c.c. of 1:1000 solutions in the treatment of severe cases of gonococcus infection of the urethra and adnexa, repeating the injections every five days for two or three times if necessary.

Shivers[194] has employed irrigations of mercurophen (1:4000 to 1:10000), preceded by irrigations with 1:8000 potassium permanganate, with uniformly good results.

Mercurochrome has been extensively employed by Young and his associates, by von Lackman and Hagen,[195] Rupel,[196] Whitman[197] and others by intravenous injection with apparently some good results. Lavandera,[198] however, injecting small doses, states that he has found the drug of no value and Brill and Myers[199] have likewise reported that mercurochrome and gentian-violet by intravenous injection did not favorably influence local and systemic gonococcus infections. Redewell and Potter[200] however, state that mercurochrome is excreted in large amounts by the kidneys and also through the prostate, seminal vesicles and other glands of the genito-urinary tract, following intavenous injections of small doses (10 to 15 c.c. of 1 per cent. solutions) every forty-eight hours. A large number of cases of acute gonorrheal urethritis, epididymitis, prostatitis and vesiculitis are stated to have improved rapidly with this medication as well as cases of acute rheumatism, synovitis, myalgia, neuritis and other manifestations of focal infection from primary foci in the prostate and seminal vesicles. To obtain the best results, they advise small enough doses to avoid too early saturation and patients showing mild reactions were observed to respond best to the treatment.

Young, Hill and Scott[201] have recently reviewed reports of four cases of cystitis and prostatitis, one of chancroidal ulcer and twenty-six of urethritis

including some of those referred to above, treated with multiple intravenous injections of mercurochrome, and believe that some cases are benefited by the maximum doses of 4 to 5 mg. per kilogram, but that urethral injections should also be used. For complications like acute gonorrheal arthritis, the results of intravenous injections have been very good; in my opinion intravenous injections of 4 to 5 mg. per kilogram should not be used routinely but employed only in the treatment of exceptionally severe infections.

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PART III

CHEMOTHERAPY OF TRYPANOSOMAL DISEASES

CHAPTER IX

PRINCIPLES OF EXPERIMENTAL TRYPANOSOMIASIS IN RELATION TO CHEMOTHERAPY

Historical.—Modern chemotherapeutic investigation may be said to have had its origin in attempts by Laveran and Mesnil[1] in 1902 to develop an efficient treatment for experimental trypanosomiasis of the lower animals. The ease with which trypanosomiasis could be produced in the small laboratory animals and the effects of treatment upon the infection determined, lead these investigators into a closer study of the effects of arsenous acid in mice, rats and dogs infected with *Tr. evansi* (surra) and *Tr. brucei* (nagana). Arsenous acid had been used for many years in the treatment of animals naturally infected with these trypanosomes but with indifferent success. For this reason Laveran and Mesnil undertook this laboratory investigation and also studied the effects of sodium cacodylate, arrhenal, mercuric chlorid, potassium iodid, certain salts of silver, quinin, formol, phenol, chinosal, salicylic acid, chloral, chlorid of lime, toluidin-blue, methylene-blue and a few other substances as well as the serums of human beings and some of the lower animals. The results were negative insofar as concerned the discovery of a superior remedy for experimental trypanosomiasis, but they were nevertheless of great importance by drawing attention to the subject of experimental trypanosomiasis as a means for determining the possible parasiticidal activity of a drug in relation to its toxicity.

In the meantime, Ehrlich had been engaged in his fundamental studies upon the affinity of dyes for cells and first applied these principles for the development of chemotherapy by employing experimental trypanosomiasis. In 1904 Ehrlich and Shiga[2] published their remarkable results with trypan red with which they were able to cure mice infected with *Tr. equinum* (caderas).

In 1903 Ehrlich had studied the effects of atoxyl upon trypanosomes in the test-tube, but the results were negative and apparently the experiments were not considered worth while continuing with experimentally infected animals since the idea prevailed then, as it erroneously does now to some extent, that an agent without parasiticidal activity in the test-tube cannot be expected to prove active in the living animal.

In 1905, however, Thomas[3] found that the injection of atoxyl and particularly mixtures of atoxyl and trypan-red, exerted some degree of curative activity in monkeys, dogs, kittens, rabbits, guinea-pigs, rats and mice experimentally infected with different species of trypanosomes (*Tr. gambiensi, Tr. brucei, Tr. equiperdum, Tr. evansi, Tr. equinum* and *Tr. dimorphon*). Sodium arsenate was found without trypanocidal activity. This work attracted the attention of Ehrlich and especially the results observed with atoxyl in *Tr. gambiensi* infections. As a result Ehrlich and Bertheim re-studied the chemistry of this remarkable substance, finding that it was not a chemically indifferent meta-arsinic-acid-anilid but an amino-derivative of phenylarsinic acid and a substance opening up wide

possibilities for the synthetic production of different combinations possessing lower toxicity and higher trypanocidal activity. At about the same time Ehrlich and Shiga (1904) studied the influence of certain dye compounds in experimental trypanosomiasis, followed in 1906 by the extensive investigations of Nicolle and Mesnil[4] with dyes of the benzidine group. These investigations and especially the work with atoxyl eventually led to the discovery of dioxydiaminoarsenobenzol (salvarsan) by Ehrlich, Bertheim and Hata, the greatest triumph in the science of chemotherapy at the present time. Atoxyl, therefore, and its humble discoverer Béchamp, are to be given the place of first importance in the history of chemotherapeutic science, and to Laveran and Mesnil belongs the credit of discovering the application of experimental trypanosomiasis for the conduct of chemotherapeutic researches.

Experimental Trypanosome Infections Commonly Employed in Chemotherapeutic Investigations.—Fortunately for the conduct of chemotherapeutic investigations, many of the trypanosomes causing disease among human beings and the lower animals are capable of infecting the smaller laboratory animals as mice, rats, guinea-pigs and rabbits. As a general rule mice have been preferred by German and French investigators and mice and rats by English and American investigators. Guinea-pigs, rabbits, and to a lesser extent dogs, goats and donkeys, have also been employed largely to supplement the tests conducted with the smaller animals.

The ability of many of these trypanosomes to infect the smaller laboratory animals has aided the development of chemotherapy in an immeasurable degree and especially in studies with the dyes and arsenicals, because these animals may be used in large numbers by reason of their cheapness and ease of handling.

As previously stated, mice and rats have been mostly employed not only by reason of their cheapness and ease of handling, but because in them trypanosomiasis is essentially an acute blood infection, a *trypanosomia*, ending fatally. It is easy to study the influence of a medicament upon the course of the disease and the results are secured in a comparatively short time. In guinea-pigs and rabbits, however, experimental trypanosomiasis is a more chronic infection of the fixed tissues; this is likewise very fortunate for experimental chemotherapy, as it permits the study of compounds under conditions more closely duplicating the course of trypanosomiasis in natural infections of man and the lower animals.

Non-pathogenic trypanosomes have proven unsuitable for chemotherapeutic investigation. Their appearance in the peripheral blood is usually intermittent and the absence of lesions and their non-fatal character leave little that is definite for the purposes of experimental chemotherapy. Furthermore some of them, and notably *Tr. lewisi*, are more resistant to trypanocidal agents than pathogenic trypanosomes. *Therefore the choice of trypanosomes for chemotherapeutic investigation is of importance* and to be carefully considered. But before discussing more fully this subject of choice of animals and organisms for chemotherapeutic investigation, it may be well to first consider certain general factors and the symptoms and lesions of trypanosomiasis produced experimentally in the smaller animals.

Natural and Acquired Virulence of Trypanosomes in Relation to Experimental Trypanosomiasis.—Extremely severe and rapidly fatal trypanosome infections are undesirable in experimental chemotherapy because slight trypanocidal effects on the part of the drug under study may become masked and readily overlooked. As a general rule, infections with a highly virulent strain of a given species of trypanosome are more difficult to influence with trypanocidal compounds than less virulent strains and for this reason the same compound tested in different laboratories, by the same method and employing the same species of organism, may yield different results.

Influence of Natural Virulence.—In the first place the period of incubation or interval of time elapsing between the inoculation and first appearance of trypanosomes in the blood or production of symptoms and the duration of life of the animal varies considerably according to the natural virulence of the various pathogenic trypanosomes. For example, *Tr. brucei* is usually more virulent for the common laboratory animals than *Tr. equiperdum*, *Tr. dimorphon*, *Tr. equinum* and *Tr. congolense* and between these extremes in varying degree are *Tr. gambiensi*, *Tr. evansi* and *Tr. rhodesiense*. The lesions and symptoms produced by the different trypanosomes in any one species of animal are remarkably similar, but the rapidity of their development and influence upon the duration of life vary in some degree, according to the species of trypanosome.

The Influence of Animal Passage upon Virulence.—Probably of even greater practical importance in relation to chemotherapeutic investigations are the changes in virulence that may be acquired by any given strain of pathogenic trypanosome, as by its "direct" passage from rat to rat or by "crossed" passage in different kinds of animals as well as by its rate of passage. Thus Thomas and Breinl,[6] working with *Tr. gambiense*, found that "if a strain is repeatedly and quickly run through animals of the same species, it will acquire a certain virulency for such a species, but this continues only so long as the strain is not run through other species of animals." However, according to Laveran,[7] passage through *guinea-pigs* does not increase the virulence of all strains; for example, in his experiments the virulence of *Tr. evansi* and *Tr. gambiense* was increased following a series of passages while the virulence *Tr. brucei* was reduced and *Tr. congolense* remained unchanged. According to other investigators, the virulence of a strain by continued direct passage may be increased for some animals and diminished for others; Martini[8] states that a strain of *Tr. brucei* run through rats and mice acquired a virulence not only for these animals but for dogs as well, whereas when passed through guinea-pigs and rabbits it became only slightly more virulent for rats, mice and dogs. I have found in my experiments that the virulence of *Tr. equiperdum* was increased by rapid passage through a series of rats but that the strain did not acquire an enhanced virulence for guinea pigs and rabbits; on the contrary passage of *Tr. equiperdum* through guinea pigs or rabbits usually reduced its virulence for rats and mice to a slight degree. Lavaran and Mesnil[9] also report that a strain of *Tr. equiperdum* which at first killed mice regularly in 5 to 10 days almost lost entirely its virulence after being kept in stock guinea-pigs for 2 to 3 months. Voegtlin, Dyer and Miller[9a] have recently reported that the virulence of a strain of *Tr. equiperdum* for rats was reduced by transfer through rabbits and a dog; curiously and probably of more importance, passage of an arsenic resistant strain through these animals resulted in a sharp reduction of this property, a subject which I will discuss with more detail in Chapter XII.

The *rate of passage* of a given strain is of considerable importance since the rapid transfer from animal to animal tends to increase the virulence. For this reason transfers at short intervals for propogating a strain should be avoided.

The *vitality of the trypanosomes and stage of the infection of the stock animal* at the time blood is drawn for transmission of the trypanosomes, also influence the character and termination of any particular infection in a given animal. The blood used for inoculation should be taken from a live or freshly killed animal and not from a dead animal since trypanosomes soon lose their infective power after the death of the host. Furthermore, blood should not be taken just before the death of the stock animal since at this time many trypanosomes may show degenerative changes and loss of motility. Moreover, as stated by Pearce and Brown, "toward the terminal stages of an infection, there are present in the blood certain 'immune substances,' the nature and amount of which are as yet little understood."

Influence of Variations in Virulence of Trypanosomes upon the Chemotherapy of Experimental Trypanosomiasis. The Arsphenamin Coefficient. During the course of trypanocidal experiments frequently conducted over a period of time as in the routine testing of the trypanocidal activity of arsphenamin and neoarsphenamin, the strain of trypanosomes (usually *Tr. equiperdum*) is apt to vary at times in resistance to these trypanocidal agents. Naturally when the resistance is markedly increased one first thinks of the possibility of acquired "fastness" through the error of employing a strain passed through animals injected with these arsenical compounds. In my experience, however, the strain may acquire a sufficient degree of enhanced virulence by passage through rats at short intervals to influence the results of trypanocidal tests. Under these circumstances the untreated controls may die one or two days earlier than usual or expected and thereby indicate that enhanced virulence may be responsible for an apparent decrease

in trypanocidal activity of the drug. On the other hand, passage of such a strain through a guinea-pig generally reduces its virulence again for rats. At any rate the trypanosomes are subject to changes in resistance that are not to be ascribed to "drug fastness" and for this reason, I have always included in each set of trypanocidal tests a lot of arsphenamin carried along in vacuum tubes from month to month. Arsphenamin does not change in trypanocidal activity when properly preserved, and serves as a control or "coefficient" for comparing the trypanocidal activity of different lots of arsphenamin, neoarsphenamin or other compounds. I shall refer to this subject of "*arsphenamin coefficient*" in the next chapter and discuss in more detail its advantages for trypanocidal tests. The adoption of this means for expressing trypanocidal values will go far toward the standardization of trypanocidal tests and give a means for comparison in different laboratories analogous to the "phenol coefficient" in bactericidal tests and the use of standard toxin or antitoxin for the titration of diphtheria and tetanus antitoxins.

The Influence of "Drug Fastness" upon the Chemotherapy of Experimental Trypanosomiasis.—Of more importance than variations in virulence of trypanosomes in relation to experimental chemotherapy is the possibility of acquired "drug-fastness." Trypanosomes readily acquire "fastness" to arsenical compounds and in laboratories conducting trypanocidal tests routinely there is always this danger unless proper precautions are taken. Trypanosomes taken from rats receiving insufficient amounts of arsphenamin or neoarsphenamin for sterilization, are usually more resistant to these compounds than a normal strain and several transfers of this kind usually results in imparting to the strain a well marked degree of resistance to the effects of arsphenamin and neoarsphenamin. For this reason animals employed for the propogation of the trypanosomes should be kept in an isolated place and removed from the possibility of becoming "fast" to the class of compounds being studied.

Variation in Natural Resistance of Trypanosomes to Therapeutic Agents. Of great importance in relation to the chemotherapy of experimental trypanosomiasis is the well known fact that some trypanosomes are more resistant to trypanocidal agents than others. For this reason an agent possessing high trypanocidal activity for one strain of trypanosome cannot be assumed to possess an equal degree of activity for other pathogenic organisms. Even different strains of the same species of trypanosome may show variations in resistance to the same trypanocidal agent, but these are not as marked as shown by different species, and sometimes are to be ascribed to variations in virulence rather than specific resistance to the therapeutic agent.

In his early experiments on the chemotherapy of experimental trypanosomiasis Ehrlich found that trypan-red was less active against *Tr. brucei* than against *Tr. equinum* in mouse infections. Laveran found that arsenic trisulfid was more active against *Tr. congolense* than against *Tr. dimorphon* in guinea-pigs. Atoxyl was found by Mesnil, Nicolle and Aubert, active against *Tr. gambiense* but only feebly for *Tr. dimorphon* and totally inactive against *Tr. congolense.* Monfort found arsenophenylglycin active in the treatment of *Tr. gambiense* from the point of view of relapses in mice, while *Tr. rhodesiense* and *Tr. dimorphon* infections were slightly more resistant and *Tr. congolense* very resistant. Mesnil and Brimont,[10] working with tartar emetic and infected mice, found *Tr. evansi* and *Tr. equiperdum* more susceptible than *Tr. brucei, Tr. equinum, Tr. gambiense* and *Tr. dimorphon* infections. More recently Yakimoff and Wassilevsky[11] found, in working with *Tr. equiperdum* infections in mice, that the ratio of the curative to the tolerated dose of luargol was 1:3 while Danysz found the ratio 1:80 in mice infected with *Tr. evansi* and 1:100 with *Tr. gambiensi.* Pearce and Brown report that in their work, *Tr. equinum* was found least resistant and that *Tr. gambiensi, Tr. brucei, Tr. evansi,* and *Tr. equiperdum* followed in the order named.

I have repeatedly found that in rats arsphenamin, neoarsphenamin and other new arsenical compounds were more active against *Tr. equinum* and *Tr. equiperdum* than against *Tr. brucei;* the results of an experiment shown in the following table brings out the variations in resistance of these three pathogenic trypanosomes. In these experiments white rats weighing from 250 to 300 grams were employed and all animals were infected in a uniform manner by the intraperitoneal injection of approximately 500,000 of the different trypanosomes, the drugs being administered by intravenous injection twenty-four hours later:

Variation in Resistance of Different Trypanosomes to Arsphenamin and Neoarsphenamin

Substance	Trypanocidal Dose per Kilo Weight			
	For Tr. Equinum	For Tr. Equiperdum	For Tr. Brucei	For Tr. Lewisi
Arsphenamin.....	0.001–0.002	0.002–0.004	0.006–0.010	more than 0.040
Neoarsphenamin.....	0.0015–0.005	0.003–0.008	0.012–0.016	more than 0.040

Apparently the variation in resistance of different trypanosomes to specific therapeutic agents is due to inherent resistance of the organisms but as will be shortly discussed, variations in resistance may be due in part at least to differences in virulence. Furthermore, as wisely pointed out by Pearce and Brown, the possibility of drug fastness should be remembered. Since so much experimental work has been done with the various laboratory strains of trypanosomes and since it is a comparatively easy matter to render a strain arsenic fast as I know from experience, it is not always possible to say whether the resistance offered to trypanocidal agents, and especially arsenic compounds, is natural resistance or one which has resulted from acquired drug fastness.

Numeric Relationship of Infection to Experimental Trypanosomiasis and Chemotherapy.—While it is necessary to use a parasite of sufficient virulence, aggressiveness and vitality to yield definite results in experimental chemotherapy, yet it is possible, as shown by the writer,[12] to mask the finer grades of therapeutic effects by an overwhelming infection, or, on the other hand, lose or vitiate an experiment by infecting with too few parasites. The time of true primary incubation, that is, the interval elapsing between inoculation and the appearance of trypanosomes in the blood, as well as to some extent the duration of the lives of the animals, are factors influenced by the numbers of trypanosomes injected. Of greater importance, as will be shortly discussed, is the fact that the parasitropic effects of a drug or substance under study and the resistance of the animal to the drugs organotropic effects are apparently modified, to some extent at least, by whether the animal (rat) is infected with small or large numbers of trypanosomes.

Because of the importance of this factor of numbers of trypanosomes injected into rats in relation to chemotherapeutic results, I have worked out a method for counting trypanosomes and inoculating with approximately known numbers, the technic being described in the succeeding chapter.

In general terms it may be stated, therefore, that the duration of primary incubation and accordingly the duration of life in experimental trypanosomiasis are influenced by the number of trypanosomes inoculated. Large differences in numbers exert these influences, small differences do not. After trypanosomes have appeared in the blood, the duration of life is about the same regardless of the numbers of organisms injected. But the numbers of trypanosomes inoculated into rats has likewise an important bearing upon the results of trypanocidal tests with various medicaments. This is especially true if the trypanocidal agent is given at an arbitrary interval after inoculation, as in the technic of my trypanocidal test in which the compound under study is administered twenty-four hours after trypanosomes have

been injected intraperitoneally. If large numbers of trypanosomes are injected a few organisms may be found next day in the blood and the amount of trypanocidal substance required for sterilization is greater than when rats are injected with small numbers of trypanosomes, as shown by Schamberg, Raiziss and myself[13] and of which the following table is an example:

THE NUMERICAL RELATIONSHIP OF INFECTION OF RATS WITH TR. EQUIPERDUM TO TRYPANOCIDAL ACTIVITY

Rat No.	Weight in Grams	Approximate Infection (Intraperitoneal)	Arsphpen. per Kilo	Examination of Blood								
				1 Da.	2 Da.	3 Da.	4 Da.	5 Da.	6 Da.	7 Da.	8 Da.	9 Da.
1	110	1,000	0.002	−	−	−	−	−	−	−	−	−
2	105	10,000	0.002	−	−	−	−	−	−	−	−	−
3	120	100,000	0.002	−	−	−	−	+	++	++++	++++	D
4	120	500,000	0.002	−	−	−	+	++	++++	D		
5	105	1,000,000	0.002	−	−	+	++	++++	++++	D		
6	115	10,000,000	0.002	−	+	++	++++	D				
7	100	100,000,000	0.002	−	+	++	++++	++++	D			

In trypanocidal tests conducted according to the Voegtlin method the influence of numerical infection upon trypanocidal values is not as important as in my method, because in the former the medicament is not administered until trypanosomes have appeared in the blood (about 100,000 to 200,000 per c. mm.). The intraperitoneal inoculation of rats with large numbers of trypanosomes simply means, therefore, that the medicament must be given earlier than when smaller numbers of trypanosomes are inoculated, since treatment is not begun in this method until examination of the blood shows the presence of the number of trypanosomes mentioned above.

Relation of Age and Weight of the Experimental Animal to the Chemotherapy of Trypanosomiasis.—In a general way young animals are more susceptible to infection with trypanosomes than older and heavier animals. This is especially true of rats, guinea pigs and rabbits. After animals are half to three quarters grown, the resistance to infection becomes more uniform, whereas in very young and old heavy animals the degree of natural resistance varies in sufficient degree to constitute a factor of variation in experimental chemotherapy.

Relation of the Route of Infection to the Chemotherapy of Experimental Trypanosomiasis.—Investigators in experimental trypanosomiasis have employed different routes of inoculation of animals. The majority have infected mice subcutaneously and intraperitoneally, rats and guinea-pigs intraperitoneally and rabbits intravenously.

The choice of route of inoculation, however, is not one of indifference although it may be influenced by convenience and technical difficulties. Infection by subcutaneous and intramuscular injection is generally followed by one or two days longer period of incubation than by intraperitoneal and intravenous injection and the animals consequently live somewhat longer. In experimental chemotherapy the administration of a medicament twenty-four hours after subcutaneous infection shows a greater influence upon the trypanosome infection than after intraperitoneal inoculation. The differences are mainly due to the fact that trypanosomes apparently do not proliferate in the subcutaneous tissues and invade the blood stream as rapidly as when inoculated intraperitoneally; consequently, fewer require destruction when a trypanocidal substance is administered twenty-four hours later.

Relation of the Route of Administration of Drug to the Chemotherapy of Experimental Trypanosomiasis.—As would be expected experiments have shown that the route of administration of a trypanocidal agent has an influence upon its trypanocidal activity, that is, upon the amount required for effecting complete sterilization. Very probably this is largely a question of rate of absorption and elimination of the medicament.

With subcutaneous injection the amounts of arsphenamin and neoarphenamin for example, required for the sterilization of rats inoculated intraperitoneally twenty-four hours previously, is usually higher than when the drugs are given intraperitoneally or intravenously; sulpharsphenamin however, may be more trypanocidal by subcutaneous or intramuscular than by intravenous injection.

With virulent trypanosome infections, as mice or rats infected intraperitoneally with large numbers of *Tr. brucei*, the subcutaneous injection of a medicament may be apparently without influence upon the infection even though the medicament is known to possess some degree of trypanocidal activity. This is due to the fact that the trypanosomes, and especially if they have been inoculated 24 hours previously, have gained too much headway in view of the slower absorption of the medicament from the subcutaneous tissues. Not infrequently when mice are employed, the medicaments are given by subcutaneous injection and in the presence of a virulent infection, there is danger that slight degrees of trypanocidal activity may be masked and hidden.

Compounds administered intraperitoneally, however, may prove trypanocidal in the same or but slightly larger doses than required by intravenous injection. This is very fortunate as it simplifies the technic in testing some compounds, but care must be exercised in the choice of compounds administered by this route as some produce so much local irritation as to kill the animal with peritonitis.

Finally it would appear proper always to give the medicament in experimental infections according to the route by which it is proposed to give it in the treatment of disease of human beings. For example, arsphenamin and neoarsphenamin employed in the treatment of syphilis are generally given intravenously and especially in the treatment of acute syphilis in the primary and secondary stages of the disease. If the compounds are injected intravenously in the experimental study of their influence upon trypanosomiasis and rabbit syphilis, they are subject to the same possible modifications in the blood stream as occurs when they are administered to human beings and this is in conformity with what should be a rule in experimental chemotherapy namely, to study the medicament under conditions approaching as nearly as possible the actual disease in human beings or the lower animals for which an efficient chemotherapy is being sought.

Time of Administration of Trypanocidal Agent in Relation to the Chemotherapy of Experimental Trypanosomiasis.—In acute trypanosomiasis of mice and rats, the results of trypanocidal tests are greatly influenced by the duration of the infection at the time the medicament is administered. This is of course to be expected inasmuch as the destruction of large numbers of trypanosomes requires more medicament than small numbers, regardless of what opinions we may entertain regarding the mechanism of trypanocidal activity.

The results are also influenced by the rapidity with which the medicament is absorbed and eliminated. For example, if a trypanocidal agent as arsphenamin or neoarsphenamin is administered intravenously to rats immediately or within a few hours after intraperitoneal inoculation of *Tr. equiperdum*, one

would expect the maximum effects but experiment has shown that this is not the case. Apparently the drug is rendered inert or eliminated too rapidly for complete sterilization when given in small amounts and is less effective against trypanosomes in the peritoneal cavity than in the blood.

In experimental chemotherapy the subject is of particular importance because if uniform results are desirable, the method must provide for the treatment of approximately equal infections from time to time. Furthermore, if the compound under study possesses but feeble trypanocidal activity, the effects may be elicited much more readily by administering the compound during light infections than by waiting until the organisms have greatly multiplied in the blood. In my experiments it was noted that when the medicaments were given within four hours of infection that sometimes more were required for sterilization than after a 24 hour period. After 48 hours however, larger and larger doses were required as the number of trypanosomes in the blood progressively increased. On the last day or two of life when the number of trypanosomes in the blood may exceed the number of erythrocytes, it is difficult to save the animal even with comparatively enormous doses of arsphenamin or neoarsphenamin.

Symptoms and Lesions of Experimental Trypanosomiasis in Mice and Rats.—Infection of mice and rats with pathogenic trypanosomes is generally an acute, progressive disease characterized by the constant presence of parasites in the peripheral blood and ending fatally. The duration of primary incubation, the interval between the inoculation and first appearance of organisms in the peripheral blood, varies with the species and virulence of the trypanosomes, the number inoculated and the route of inoculation.

As a general rule trypanosomes are to be found in the blood in from twenty-four to forty-eight hours after intraperitoneal injection (the usual route of inoculation) and from then on multiplication is rapid until death, when they seem to be more numerous than the red corpuscles. Occasionally the course of *Tr. equiperdum* infections is interrupted for a day or two by a slight remission in the number of organisms in the blood. *Tr. equinum* infection may also run a relapsing course, especially in mice, the parasites being greatly diminished in number on the fourth or fifth day followed by rapid increase and death on the tenth to fourteenth day after inoculation.

The animals are remarkably free of symptoms up to the time of death. During the last few hours they are apt to be quiet and drowsy followed by the development of tremors, violent convulsions and death. On the other hand, death may follow in a state of coma without convulsions. Laveran and Mesnil have stated that mice infected with surra, caderas and dourine become very quiet and sit huddled up with a rough and bristling coat, eyes closed, and insensitive to external stimuli; just before death the corneas may become white and opaque.

Symptoms and Lesions of Trypanosomiasis in Guinea Pigs and Rabbits.—Experimental trypanosomiasis in guinea-pigs and rabbits constitutes a type of infection in sharp contrast to trypanosomiasis in the mouse and rat. Instead of an acute and rapidly fatal blood infection, the parasites appear in the blood only at intervals and chronic or subacute involvement of the fixed tissues becomes a more conspicuous and predominating feature. Pearce and Brown have given an excellent description of the disease in these animals.

The Application and Utilization of Experimental Trypanosomiasis in the Development of Chemotherapy.—Experimental trypanosome infections of the lower animals have proved of great value in the study of the principles of chemotherapy, that is, the selective action of chemical substances for microparasites in living hosts. The large size of these organisms, the ease by which laboratory animals may be infected and the course of the infection studied, the fatal character of the infection with pathogenic trypanosomes and the cheapness of mice and rats, have been some of the reasons why experimental trypanosome infections have commanded so much attention and

investigation. Experimental chemotherapy is usually a very expensive type of investigation; large numbers of animals are required and the time and labor involved are tremendous.

Choice of Animal.—As previously mentioned trypanosomiasis in mice and rats is an acute blood infection without much involvement of the fixed tissues. Trypanosomiasis of guinea-pigs and rabbits is largely one of infection of the fixed tissues in which the blood is invaded only at intermittent periods. In mice and rats, the disease is usually rapidly fatal and the problem of treatment analogous to acute bacterial infections with bacteremia; in guinea-pigs and rabbits the diseases is chronic and may run months and even years.

For determining *quickly* the trypanocidal effect of a drug, mouse and rat infections are to be preferred but the information yielded may not have a direct application in the treatment of trypanosomiasis in human beings, horses and cattle. In the latter the question of tissue involvement is more important than blood invasion and compounds like arsphenamin and neoarsphenamin, possessing extremely high trypanocidal activity in mouse and rat infections, have less effect in guinea-pig and rabbit infections and a great deal less in the treatment of trypanosomiasis of human beings, horses and cattle. Mouse and rat tests are splendid for preliminary orientation—for learning quickly whether or not a compound under study possesses trypanocidal activity, but for securing data on its probable value in the treatment of human trypanosomiasis and natural infections of horses, cattle and other of the larger animals, tests on guinea-pigs and rabbits are required in order to study the drug in the presence of chronic tissue infections where passage of the drug into the pathological lesions and healing of the latter are questions of prime importance.

As between the choice of mice or rats for the acute blood infections, I think preference should be given the albino rat of 100 to 200 grams of weight. Breinl and Nierenstein[14] state that their reaction is more constant than mice, likewise their resistance to infection. They are very susceptible to trypanosome infections, are cheap and easy to handle (with gloves). Furthermore, in my experience, it is easier to inject them intravenously and intravenous injections are generally employed in experimental chemotherapy. On the other hand, Dale and White[15] have preferred mice for conducting the official toxicity and trypanocidal tests of arsphenamin and neoarsphenamin in England; their associates Burn, Durham and Marchal found that the results were practically the same as observed with rats, that mice were easier to handle and may be injected intravenously with less time and labor.

As between guinea-pigs and rabbits for chronic tissue infections, I believe that preference should be given rabbits of about 1500 to 2000 grams, and preferably male animals. They may be infected by either intravenous or intraperitoneal injection and repeated intravenous injections of medicament are given with great ease. Furthermore, it is easier to examine the blood repeatedly and the tissue changes are more definite and detectable.

Choice of Parasite.—The majority of chemotherapeutic investigations in experimental trypanosomiasis have been conducted with *Tr. brucei.* Infections with this parasite and *Tr. evansi* are usually highly virulent, and rapidly invasive. *Tr. equiperdum, Tr. gambilnsi* and *Tr. equinum* are less virulent and less rapidly fatal.

For general chemotherapeutic work I prefer *Tr. equiperdum* infections in rats and *Tr. brucei* infections in rabbits. *Tr. brucei* infections of rats are usually too virulent and rapidly fatal with the likelihood of masking the feeble but definite trypanocidal effects of some drugs. On the other hand, in rabbits *Tr. brucei* infections are better than *Tr. equiperdum* infections because the symptoms and lesions are usually more marked.

For specific chemotherapy of a certain infection, the trypanosome of that disease should be employed in the final experiments and the laboratory animal should be one in which the trypanosome produces lesions approaching in character those actually produced in the natural disease. For example, in work upon the chemotherapy of human sleeping sickness, *Tr. gambiensi* should be used in rabbits for the final tests of a drug; in dourine of horses, *Tr. equiperdum* infections in rabbits should be used. In other words, our final efforts should be made under conditions approaching the actual or natural disease as closely as possible—using rabbits infected with the specific organ-

ism in order that the particular parasite may be attacked in the chronic tissue lesions, in the spinal fluid and as they occur intermittently in the blood.

Application of Experimental Trypanosomiasis in the Chemotherapy of Syphilis and Other Spirillar Infections.—Investigations in the chemotherapy of experimental trypanosomiasis have had their greatest *practical* value and application in the development of the treatment of syphilis. Ordinarily it may be said that a chemical compound possessing a high and specific parasitropism for trypanosomes may not possess a similar destructive affinity for such organisms as *Spirochaeta pallida*, *Spirillum obermeieri* and other pathogenic spirilla. Indeed, opinion has leaned toward the assumption that no reliable comparison could be drawn between the trypanocidal effects of a drug in rats or mice and spirocheticidal effects in human beings or rabbits.

Many of the organic arsenical compounds, however, and especially arsphenamin and neoarsphenamin, have proved both trypanocidal and spirocheticidal. This fact has greatly aided in testing the curative activity of arsphenamin and neoarsphenamin because there is a close parallelism between trypanocidal and spirocheticidal activities insofar as the organic arsenicals are concerned. For example, I have determined the smallest trypanocidal dose of several different brands of arsphenamin and neoarsphenamin according to my method described in the succeeding chapter. Rabbits were infected intratesticularly with *Spirochaeta pallida* and treated with varying amounts of the different arsphenamins and neoarsphenamins by intravenous injection as soon as the testicle presented the lesion of syphilis and numerous spirochetes when examined microscopically. With rabbits it was determined for each lot of arsphenamin and neoarsphenamin the minimal amount required to not only cause the disappearance of all organisms from the testicular lesions but likewise from the neighboring lymph glands, which were emulsified and injected into fresh animals. The results of my experiments have shown that arsphenamin was trypanocidal in 3 to 4 mgm. per kilogram of rat and spirocheticidal in 8 to 12 mgm. per kilogram of rabbit. In general there was parallelism between the two values. For example, an arsphenamin trypanocidal in dose of 4 mgm. per kilogram was spirocheticidal in 8 mgm.; a second arsphenamin was trypanocidal in 6 mgm. and spirocheticidal in 12 mgm.; a third arsphenamin was trypanocidal in 3 mgm. and spirocheticidal in 10 mgm. For arsphenamin therefore, the relation was quite constant, the trypanocidal dose being about one half the spirocheticidal dose, that is, the relation being 1:2 to 1:3.3.

With neoarsphenamin the relation between trypanocidal and spirocheticidal was less constant, the trypanocidal dose being from 1.3 to 6 times less than the spirocheticidal dose. For example, one lot was trypanocidal in dose of 6 mgm. per kilogram and spirocheticidal in 8 mgm.; another lot was trypanocidal in dose of 2 mgm. and spirocheticidal in 12 mgm. etc.

Clinical evidence of a parallelism between trypanocidal and spirocheticidal activities of arsphenamin and neoarsphenamin has been furnished by the investigations of Mills and White,[16] who determined the minimal trypanocidal dose of a number of compounds of British manufacture employing white mice infected with *Tr. equiperdum*. Compounds showing different trypanocidal activities were then selected and administered in dose of 0.45 gm. by intravenous injection to human beings with syphilis, care being taken to select primary cases with chancres most nearly approaching a clinical uniformity in order to minimize error due to varying degree of rate and amount of penetration of the drug into the lesions. The lesions were then examined for spirochetes 18–20 hours later and these investigators concluded that the correspondence between the two values was so good as to justify the conclu-

sion that the determination of the therapeutic action on mice infected with trypanosomes is a very valuable index, if not an accurately quantitative measure, of the therapeutic activity of different samples of neoarsphenamin for syphilis in man. The method has, accordingly been put into routine use in the Medical Research Council's Department of Biological Standards in England, an occasional check of therapeutic activity being thus imposed, in addition to the regular testing of every batch of such products for absence of undue toxicity.

It cannot be said, however, that all organic arsenical compounds possessing trypanocidal activity have an equal degree of spirocheticidal power; for example, tryparsamid, the sodium salt of N-phenylglycineamide-p-arsonic acid, prepared by Jacobs and Heidelberger[17] and tested biologically by Brown and Pearce, possesses a very high curative activity against many different pathogenic trypanosomes but much less effect upon *Spirochaeta pallida* and especially in the treatment of human syphilis.

In a general way, however, the majority of organic arsenical compounds and some dye substances have shown both trypanocidal and spirillocidal activities. Most of them have proven more highly spirillocidal than trypanocidal insofar as tests employing *Sp. gallinarum* (fowl spirillosis) and *Sp. obermeieri* (relapsing fever) are concerned. Most of Ehrlich and Hata's early work was done with these spirilla. Some compounds possessing feeble trypanocidal activity were found actively spirillocidal indicating that fowl spirillosis and mice infected with *Sp. obermeieri* are more easily cured than mice or rats infected with trypanosomes.

Since trypanosomes may be more resistant to destruction by chemical agents than spirilla, a compound unable to influence a trypanosome infection may yet demonstrate curative activity for spirillar infections like *Sp. obermeieri* infections of mice, *Sp. gallinarum* infections of fowls or *Spirocheta pallida* infections of rabbits. Therefore, *trypanocidal tests may be said to possess a greater positive than negative value and especially in the experimental chemotherapy of spirillar diseases*; that is to say, a drug without demonstrable trypanocidal activity may still prove spirocheticidal. In this connection special mention should be made of mercurial compounds. Even in amounts equivalent to their maximum tolerated doses they are without or almost without demonstrable trypanocidal effect and yet the experience of centuries has demonstrated their curative activity in the treatment of human syphilis.

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CHAPTER X

TECHNIC OF TRYPANOCIDAL TESTS

As discussed in the preceding chapter there are many biological and technical factors to be considered in conducting trypanocidal tests. The choice of animal and infecting trypanosome, the method of inoculation, the time interval between infection and administration of the compound under study and the route of injection of the latter, are the more important of these biological and technical factors. The simple infection of a series of rats or mice with a pathogenic trypanosome followed by administration of the compound and a subsequent study of its effects if any upon the infection, are relatively simple technical procedures, unless a maximum degree of accuracy and uniformity of results are required, in which case technical details require the closest attention.

Requirements of an Acceptable Trypanocidal Test.—In the majority of instances trypanocidal tests are required for the purpose of determining whether or not a chemical substance possesses trypanocidal activity; and if so, a quantitative measure of this property. Tests of this kind are being routinely employed at present in this country and England for testing the curative activity of arsphenamin, neoarsphenamin and allied compounds, since, as stated in the preceding chapter, a measure of this property is acceptable as an index of spirocheticidal activity insofar as these arsenical compounds are concerned. Rat and mouse infections are employed in order that the results may be obtained as quickly as is consistent with accuracy and with the minimum expense and labor. When it is desired to study compounds for the treatment of human trypanosomiasis or trypanosomiasis of the lower animals (particularly horses and cattle), experimental guinea pig or rabbit infections are advisable in addition to rat or mouse tests, in order that the medicament may be tested for curative activity in the presence of chronic tissue infections. These guinea pig and rabbit tests are also advisable in studies with compounds to be used in the treatment of syphilis, because the analogy between rat trypanosomiasis and human syphilis holds good only for the early stages of syphilis, when spirochetes are present in the blood and lymph and before marked tissue changes have occurred; whereas the tissue changes of chronic and latent syphilis bear a closer analogy to the pathological tissue changes in human trypanosomiasis and to experimental trypanosomiasis of guinea-pigs and rabbits. It may be said in this regard that the chemotherapy of acute primary syphilis has been solved with arsphenamin and neoarsphenamin; that the results are almost as satisfactory as the treatment of trypanosomiasis of rats and mice, but this does not apply to the treatment of cases of long standing chronic syphilis with tissue changes. For the development of a more satisfactory chemotherapy of syphilis in its latent and tertiary stages, guinea-pig and rabbit trypanocidal tests and treatment of rabbit syphilis are probably just as important as rat or mouse trypanocidal tests.

The requirements of an acceptable trypanocidal test may be summarized as follows:

1. *It should yield quantitative results in an accurate and consistent manner* so that the measure of trypanocidal activity may be expressed in milligrams of compound per gram of body weight of animal.

2. *The same compound tested at different times should yield the same or approximately the same results, providing it is known that chemical changes in the compound likely to alter trypanocidal activity have not occurred.* Since arsphenamin is known to maintain its activity in a uniform manner over long periods of time I have checked up my technic routinely with this substance and for several years have compared the trypanocidal activity of other substances with arsphenamin in what has been designated as the "arsphenamin coefficient."

3. *It should be as economical as possible in both animals and labor.* Chemotherapeutic research is expensive—probably the most expensive of all medical research. Ordinarily many experiments requiring large numbers of animals and much time and labor are required for the thorough study of any compound. For routine tests to pick out the "leads" or more promising compounds, the test should be economical and yield reliable information as quickly as possible. Elaborate and time consuming tests are uncalled for and unnecessary. The simpler the technic the better are the results, providing fundamental principles are observed. For routine tests rat and mouse infections are satisfactory when conducted with a pathogenic organism as *Tr. equiperdum* or *Tr. brucei;* for special tests involving the question of treatment of chronic tissue lesions, guinea pig or rabbit tests are demanded. Likewise trypanocidal tests for the development of the chemotherapy of a special trypanosome disease should employ if possible the organism of that particular infection, as *Tr. gambiensi* in chemotherapeutic investigations or African sleeping sickness.

Propagation of Trypanosomes.—Pathogenic trypanosomes are propagated by injecting blood from an infected animal into stock animals at suitable intervals. *Due care must be exercised against inoculating too many trypanosomes at too close intervals, because this practice sometimes results in greatly enhancing the virulence of the organisms.*

As a general rule, trypanosomes should be propagated in both rats and guinea-pigs, the former for trypanocidal tests employing rats and the latter for tests employing guinea-pigs and rabbits. Furthermore rats succumb more quickly and a strain may be lost, whereas guinea pigs live for four weeks or longer from which the strain may be recovered at intervals, when parasites are present in the blood. As stated above, *trypanosomes employed in rat tests should be propagated in rats; if at any time a strain is taken from a guinea-pig or rabbit it should be passed at least twice through rats before being used in rat tests in order* to restore virulence to the uniform level for rats. In guinea-pigs the virulence is reduced and as stated in the preceding chapter, it is my custom to pass strains through one or two guinea-pigs, if the virulence for rats becomes higher than usual. The methods employed by Pearce and Brown[1] have proven very satisfactory.

It is a good rule to always inject several animals (mice, rats or guinea-pigs) with varying amounts of blood suspension in order that the animals may not all die at approximately the same time and so make the recovery of the strain uncertain.

Author's Method for Conducting Trypanocidal Tests Employing Rats.—This method[2] was developed in 1914–1915 in connection with chemotherapeutic investigations conducted in coöperation with Dr. Jay F. Schamberg and Dr. George W. Raiziss. It has been in routine use in my laboratory since then and in our work has proven reliable and satisfactory. It yields remarkably uniform and decisive results with a minimum of expense, labor and time and has fulfilled the essential requirements of a routine trypanocidal test.

The Trypanosome.—After considerable experience with *Tr. brucei*, *Tr. equinum* and *Tr. equiperdum* the last mentioned has been found the most useful and satisfactory. *Tr. brucei* infections of rats are ordinarily too severe and sometimes mask feeble but important trypanocidal effects of some compounds. A strain of *Tr. equiperdum* maintained at moderate virulence permits rats to live about six days and this is a sufficiently severe infection. The test may be conducted with any of the pathogenic trypanosomes but

my preference is for *Tr. equiperdum.* If the strain acquires too much virulence by means of a rapid succession of transfers, virulence may be reduced by passing the strain two or three times through guinea pigs or rabbits.

The Animal.—Male albino rats weighing from 150 to 200 grams are employed. The diet is probably not of as much importance as in toxicity tests (see Chapter II), but of course the animals must be in good health and general condition. It is my rule to have the rats under observation for at least a week before employing them in these tests.

Old and heavy animals are sometimes too resistant and the weights of the animals should be approximately uniform, that is, not less than 100 grams or more than 200 grams. Male rats are preferred because female rats of 150 or more grams may be pregnant at the time of the tests and receive a larger dose than they should according to body weight.

Method of Inoculation.—Approximately known numbers of trypanosomes are infected intraperitoneally 24 hours before the medicament is administered. For rats of 100 to 200 grams the number may be 250,000 to 500,000 trypanosomes. *It is particularly important in this method to infect the animals uniformly because the medicament is given arbitrarily 24 hours later;* if the infection is too light or too heavy the results are influenced. In the Hygienic Laboratory method of Voegtlin and Smith numerical infection is much less important because each rat is examined and the trypanosomes counted before the medicament is given.

The counting of trypanosomes and technic of inoculation[3] are as follows:

Seed Animal.—For the purpose of making an accurate count, the seed animal should be one that has not been infected over three or four days as after this time the parasites in the peripheral blood may be very numerous and, what is more important, may be gathered in conglomerated masses which cannot be broken up in the mixing pipet and which render any attempts at counting absolutely worthless.

A preliminary examination of the blood is first made in the usual manner by snipping off the tip of the tail and quickly transferring a drop of the blood on a clean cover glass to a microscopic slide. The weight of the glass is allowed to spread the blood. This preparation is then examined by means of a No. 1/6 objective and No. 4 eyepiece (Leitz), and the number of trypanosomes recorded according to the following scheme:

++++ = very large numbers; cannot be counted.
+++ = large numbers that may be roughly counted (not accurately).
++ = about 10–20 in a field.
+ = about 5–10 in a field.
Few = 1–2 in a field, or in every other field.

This preliminary examination is necessary because it indicates the blood pipet to use, as the red corpuscle pipet for a 1:200 dilution with + + + + and + + + rats, and the white corpuscle pipet for a 1:20 dilution with + + and + rats. This method of recording the count of trypanosomes has been found useful as a routine procedure in the daily examination of large numbers of rats in our chemotherapeutic researches and with due care yields a satisfactory record.

Diluting Fluid.—After some experimentation a fluid has been adopted which serves the three-fold purpose of (1) fixing the trypanosomes and rendering them motionless, (2) quickly staining the trypanosomes so that they are readily seen and counted, and (3) removing the erythrocytes by hemolysis so that the parasites are readily seen in a clear field. It is easily prepared and comparatively stable, remaining clear, without sediment, for

many weeks and months at ordinary room temperature. This fluid is prepared as follows:

Formalin (40 per cent.)	2 c.c.
Glacial acetic acid	2 c.c.
Distilled Water	96 c.c.

Mix and add 2 c.c. carbolfuchsin (Ziehl-Neelson); mix again and filter through paper. This quantity of fuchsin in the presence of formalin gives the fluid a clear, deep-purplish red color. Probably less fuchsin will prove satisfactory to others; more of the dye deepens the color of the fluid unnecessarily and may somewhat obscure the lines of the counting chamber. The formalin quickly destroys the motility to trypanosomes and keeps the fluid free of bacteria and fungi. The container should be well stoppered to prevent evaporation, and it may be advisable to refilter the fluid at occasional intervals.

Voegtlin and Smith[4] state that this fluid sometimes causes a coagulum of unlaked red cells and trypanosomes and they prefer a slight modification prepared by dissolving 0.02 gm. of crystal violet in 200 c.c. of water and slightly warming; 0.7 gm. of sodium chlorid is added, the solution cooled and filtered. Formalin is added (2 c.c. in 200 c.c.) to fix the trypanosomes and prevent their loss of shape. This fluid should be freshly prepared as required.

Counting of the Trypanosomes.—After the seed animal has been selected a count is made of the trypanosomes. The tail is gently whipped back and forth for a minute or two to produce congestion; then, upon very gentle pressure, a large drop of blood appears where the tip has been snipped off. With the red corpuscle pipet, blood is carefully drawn to the mark 0.5 and the diluting fluid to the mark 101, the whole procedure being conducted with the care and exactness required for making an erythrocyte count. If the white corpuscle pipet is used, a large drop of blood is required and hence, before attempting to fill the pipet a free flow of blood must be assured. With this pipet, blood is drawn to the mark 0.5 and the diluting fluid to the mark 11, as in the usual technic for counting leukocytes. As a general rule, the red blood corpuscle pipet is indicated and is more satisfactory than the white blood corpuscle pipet.

The filled pipet is now thoroughly shaken for at least one or two minutes; several drops are expelled, and then sufficient fluid is placed in the chamber and the cover glass accurately adjusted. The slide is allowed to rest on a flat surface for at least ten minutes to permit the trypanosomes to sink down on the ruled off spaces.

By means of the No. 1/6 objective and No. 4 or No. 2 eyepiece (Leitz) and a mechanical stage, all the trypanosomes in the ruled off space (400 small squares) of the Thoma ruling are counted. The trypanosomes are motionless, deeply stained a purplish red, and easily seen and counted. At times they are curled or placed edgewise, but with a clean diluting fluid, to avoid artefacts, and a little experience these forms are readily recognized (Fig. 7). The red blood corpuscles have entirely disappeared, or appear as faint shadows. The leukocytes also are deeply stained, and a total leukocyte count may be made at the same time. A differential leukocytic count is also possible, at least as far as differentiating between the polynuclear and mononuclear leukocytes.

Calculation of the Number of Trypanosomes in a Cubic Centimeter Undiluted Blood.—As each square contains 1/4000 cubic millimeter when the cover glass is properly adjusted, it is necessary to count the trypanosomes in 4000 small squares to obtain the number contained in 1 cubic millimeter

of the diluted blood. By counting the trypanosomes in 400 small squares and multiplying by 10, the approximate number in 4000 small squares, or 1 cubic millimeter, is obtained. By multiplying this figure by the dilution, as 200 if the red blood corpuscle pipet was used, or by 20, in case the white blood corpuscle pipet was used, the number in 1 cubic millimeter of undiluted blood is obtained. Multiplying this figure by 1000 gives the approximate number of trypanosomes in a cubic centimeter of undiluted blood.

Briefly, by rule of thumb, count the trypanosomes in 400 small squares, multiply by 200, and add four ciphers. The result is the approximate number of trypanosomes in a cubic centimeter of undiluted blood.

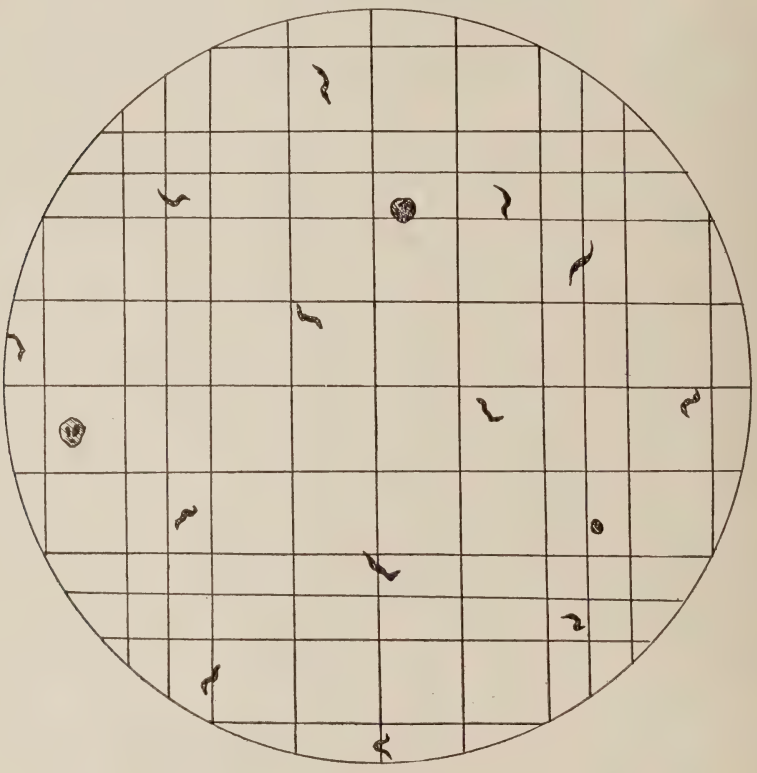

FIG. 7.—METHOD FOR COUNTING TRYPANOSOMES IN THE BLOOD.

A section of the four hundred small squares of the Thoma ruling, showing trypanosomes in blood, diluted 1:200 (182,000,000 per cubic centimeter of undiluted blood), from a ++++ rat infected with *Trypanosoma lewisi*. × 288 (Leitz No. 6, eyepiece No. 2).

If the white corpuscle pipet is used, the calculations are the same, except that the dilution is 1:20. By rule of thumb, count the trypanosomes in 400 small squares, multiply by 20, and add four ciphers.

A question of considerable importance in this connection is whether counts of trypanosomes made with blood from the tail may be accepted as reliable and similar to counts made with blood from the heart, especially as blood for the purpose of transmitting the parasites is obtained from the heart of the seed animal? I have made a series of counts of blood obtained from the tail and from the right and left ventricles of the heart; it was observed that the counts ran fairly parallel, especially in view of the large number of

trypanosomes present and the chances of manipulative error. Frequently counts of blood obtained from the left ventricle showed slightly fewer trypanosomes than those of blood from the right ventricle and tail, but on the whole they were sufficiently close for the purpose of counting trypanosomes in the blood from the tail and using this figure as representing the number present in blood from the heart in the transmission of approximately known numbers of trypanosomes. It must be emphasized, however, that blood from the tail must flow easily and freely before counts are reliable; those from blood obtained after squeezing and massage of the tail are inaccurate and unreliable.

Securing of Infected Blood for Transmissions.—Blood is obtained from the heart of the seed animal. In this way, 0.1 c.c. of blood may be obtained in

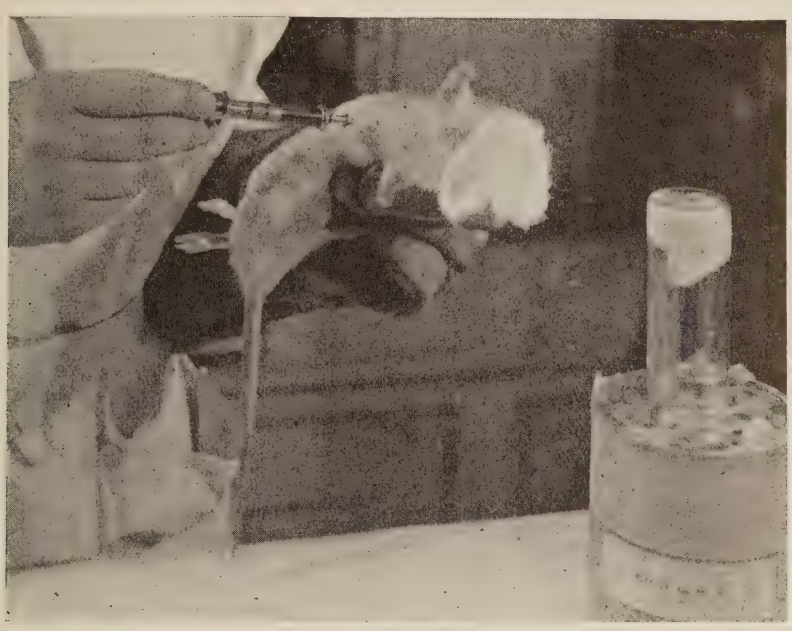

FIG. 8.—METHOD FOR REMOVING BLOOD FROM THE HEART OF A RAT.

an accurate and sterile manner, the success of this method of transmitting known numbers of trypanosomes depending of course upon the operator's success in obtaining the blood accurately and quickly from the heart. There must be no more and no less blood, and the dilutions must be accurate.

This amount of blood is easily obtained from the living rat. If it cannot be drawn accurately, the animal should be killed, the thorax opened, and the blood drawn directly from the heart under the eye of the operator. With a little practice, however, one seldom fails to draw the blood from the living rat, and usually the operation does not appear to injure the animal.

It is necessary to use an accurate and well adjusted 1 c.c. syringe divided into tenth cubic centimeters. I prefer the Record syringe fitted with a No. 22 needle. A proper needle is very important; it must be sharp and sufficiently large to permit the easy flow of blood and yet not so large as unnecessarily to injure the heart.

(1) Warm a test tube containing 10 per cent. sodium citrate in normal salt solution in a Bunsen burner and draw up enough of this into the syringe

to make, when air has been expelled, 0.4 c.c. including the needle. (The sodium citrate prevents coagulation of the blood under isotonic conditions.)

(2) The rat being held by an assistant, or fastened to an operating board, paint the left thorax with tincture of iodin and quickly insert the needle at about the point of maximal pulsation (Fig. 8). By gentle suction on the piston, blood is made to flow into the syringe. If blood does not appear, re-insert the needle in an upward and inward direction without withdrawing it entirely. Draw blood accurately to the 0.5 c.c. mark (=0.1 c.c. blood). Withdraw the needle and draw more of the warm citrate solution to the 1 c.c. mark. This gives a dilution of blood in the syringe equal to 1 in 10. Shake gently in order to mix the blood.

(3) Expel the contents of the syringe into a test tube containing 9 c.c. of warm salt solution and wash out the syringe three or four times with this solution, or the contents of the syringe may be expelled into a tube containing 6 c.c. of warm normal salt solution and the syringe washed out with 3 c.c. fresh warm salt solution into this tube in order better to remove all trypanosomes from the syringe and needle. This gives a suspension of 1:100, and from this tube further suspensions of the trypanosomes may be made in warm, sterile salt solution as required.

If one fails to secure blood in this manner it may be collected more easily as follows:

In an accurately graduated 25 c.c. cylinder place 10 c.c. of warm sodium citrate solution. Sever the vessels of the throat of the animal with sharp scissors and collect the blood by means of a small funnel. Read off the amount of blood obtained and dilute with warm physiological saline solution as required.

Dilution of Infected Blood for Transmission.—A 1:100 suspension of infected blood having been secured, further suspensions may be made by transferring 1 c.c. to a series of test tubes containing 9 c.c. of warm, sterile salt solution. The 1:100 suspension and each succeeding one should be thoroughly mixed by furnishing each test tube with a sterile rubber stopper and then gently agitating the contents with turning up and down until a thorough admixture results. With the final suspensions, a series of rats may be infected by injection of each intraperitoneally with a cubic centimeter or fraction thereof, according to the count.

In conducting chemotherapeutic researches with *Tr. equiperdum*, best results have been obtained when the rats were infected with about 100,000 trypanosomes per animal. With *Tr. brucei* fewer may be used and with *Tr. lewisi* a larger number should be injected.

Method of Infecting Rats.—Rats may now be infected by the intraperitoneal injection of the proper dose of trypanosome suspension. For this purpose, I prefer a 1 c.c. Record syringe fitted with a No. 22 needle. The suspension of trypanosomes should be briefly and gently mixed each time before the syringe is loaded in order to avoid settling. The syringe should be sterile and the technic fairly aseptic, although I have never seen infection of the peritoneum follow when the rat was properly entered. I have not found it necessary to clip the hair or sterilize the skin by washing or even by an application of tincture of iodin, and with due care the intestines are not punctured.

Time Interval between Inoculation and Treatment.—Twenty-four hours later the medicament is administered. During this time trypanosomes have multiplied and an occasional parasite may be found in the tail blood examined microscopically. If rats of approximately the same weight are inoculated with uniform numbers of trypanosomes the resulting infection is

quite regular although the natural resistance of individual animals influences the rate of multiplication and invasion of the blood stream. Brown and Pearce also inject the medicament 18 to 24 hours after intraperitoneal inoculation whereas Voegtlin and Dale infect the animals more heavily and wait until the blood stream has been more extensively invaded with trypanosomes, before treatment is begun. There is however, no essential difference in the end results except that the curative dose in my method is less than in these other methods because fewer trypanosomes require destruction. This subject will be discussed at greater length later on.

Intravenous Injection of Drug.—The rats are kept fasting for at least 12 hours prior to treatment. Each animal is carefully weighed, numbered and recorded. The compound for treatment is prepared in solution and administered according to the body weight of the respective animals. Ordinarily 4 rats are used for treatment with varying amounts of drug and 2 rats kept as untreated controls. Or 4 controls are used for a series of 20 to 40 treated animals.

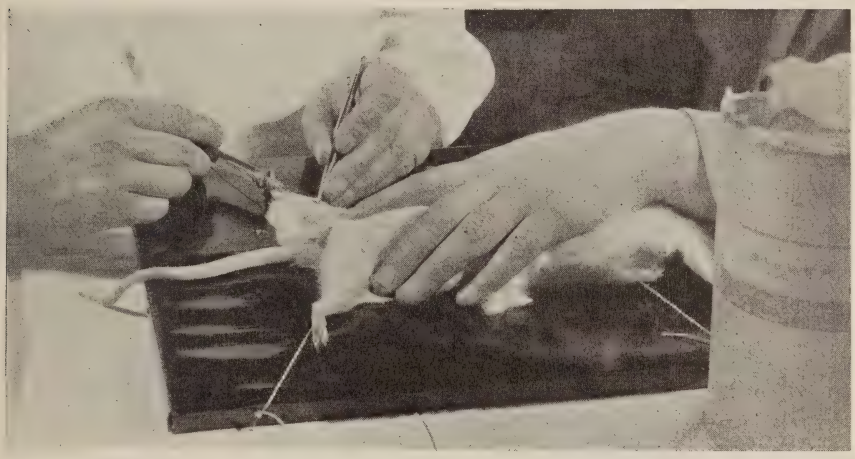

FIG. 9.—METHOD FOR INTRAVENOUS INJECTION OF THE RAT IN TRYPANOCIDAL TESTS EMPLOYING THE FEMORAL VEIN.

The medicament is prepared for each animal in such way that the amount of fluid injected is 1 c.c. Smaller amounts are unadvisable because of the influence upon results if a drop or two is lost in the process of injection.

The injections are given intravenously, the saphenous vein is generally employed, the technic being as follows:

(*a*) The rat is tied on its back to an operating board. This is a simple board covered with white oil cloth for facilitating cleansing and furnished with nails at the four corners for tying the animal. An assistant wearing gloves handles the animal and by means of slip knots the animal is quickly and securely tied.

(*b*) A wad of cotton is now placed under one thigh to render the tissues firm. The assistant places his finger in the inguinal region to make pressure and render the saphenous vein more prominent.

(*c*) The skin is wiped with alcohol and a small incision made at about the middle of the thigh and in line with the vein (Fig. 9). With a little dissection the vein is brought into view and is grasped with a fine pointed pair of forceps and steadied. A suture of black silk may be placed below the point

selected for injection in order to prevent subsequent bleeding. This is advisable when neoarsphenamin is being injected because of the lowered coagulation time of the blood, but otherwise it is not necessary as bleeding usually ceases as soon as the rat is released providing a No. 26 needle has been used and the vein not mutilated with several puctures.

A 1 c.c. syringe fitted with a No. 26 needle is employed, being prepared beforehand and in readiness. The eye of the needle is slowly inserted bevel upwards; the assistant releases pressure in the inguinal region but holds the leg against movement and the injection is slowly given. The vein is now tied off if neoarsphenamin has been injected and the rat is quickly released. With experience an operator and assistant can easily inject 20 rats in an hour.

The wound is not sutured; infection practically never occurs unless some of the solution and especially arsphenamin and neoarsphenamin have been injected in the tissues around the vein and necrosis thereby produced.

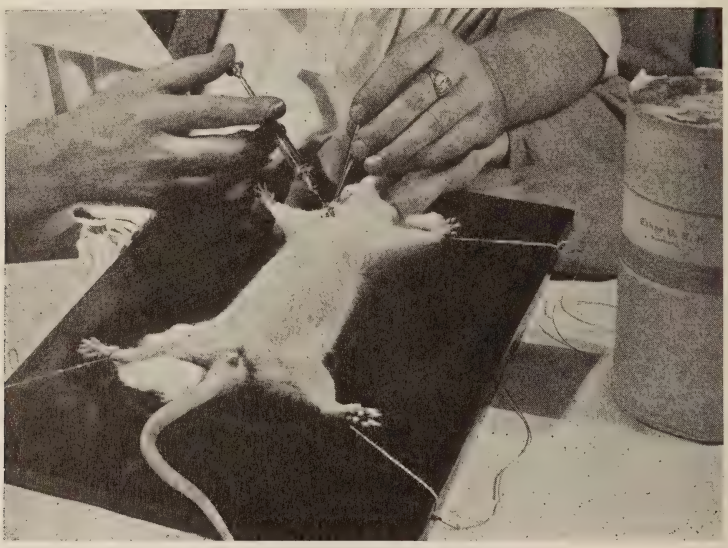

FIG. 10.—METHOD FOR INTRAVENOUS INJECTION OF THE RAT EMPLOYING THE EXTERNAL JUGULAR VEIN.

When the injection has been cleanly and expertly given, healing occurs promptly and without infection.

An *external jugular vein* may be used instead and has the advantage of being larger. For several years I employed one of these veins with uniform success but I now believe that the saphenous vein is to be preferred because there is less bleeding; this is especially true when neoarsphenamin is administered.

The animal is tied to the operating board in the same manner and a wad of cotton placed under one shoulder. The assistant holds the head backwards and to one side with a piece of cotton rendering the tissues of the operative area tense and firm (Fig. 10).

The skin is touched with alcohol and a small incision made just above and about in the center of the area where a clavicle in the human would be situated. The subcutaneous tissues are gently dissected and the vein exposed which becomes very prominent with respiratory movements. It is well not to attempt entering the vein until it is thoroughly exposed as other-

wise one may infiltrate the tissues over and about the vein by failure to enter it properly. As soon as the injection has been given the animal is quickly released and the wound requires no attention as infection practically never occurs and healing is rapid.

Rats and mice may be injected by means of a caudal vein of the tail described in Chapter II. This method is practically the only one employed for the intravenous injection of mice. The veins are quite small, and the injection requires a fine needle and considerable experience.

Study of Effects;—"Minimal Effective Dose" and "Minimal Curative Dose."—The control and treated animals are kept under observation and blood from the tail of each examined daily. *Each animal is kept in an individual cage* because when a group of rats are kept together the stronger and healthier may annoy and finally destroy the weaker and sicker animals. Furthermore treated rats recovering from infection may later become

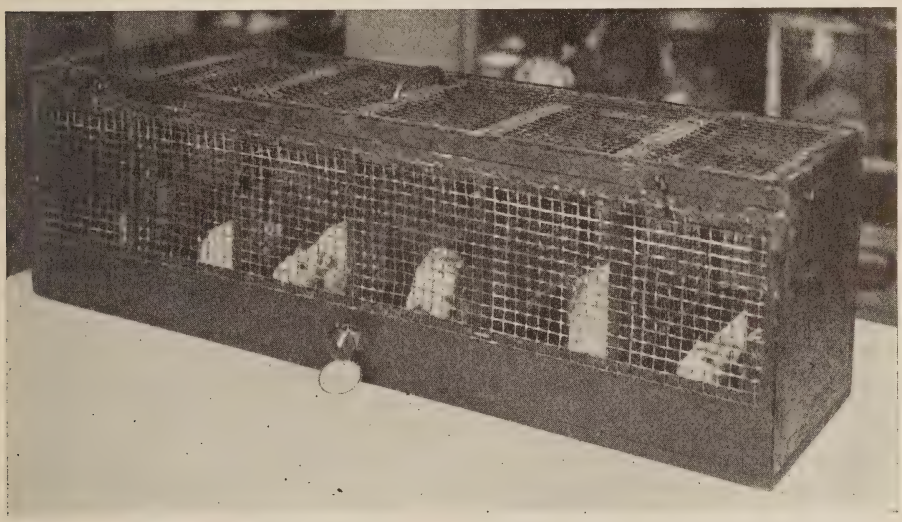

FIG. 11.—CAGE FOR KEEPING RATS SEPARATELY IN TOXICITY AND TRYPANOCIDAL TESTS.

infected by eating rats with trypanosomiasis and this tends to introduce an element of error and irregularity in the results. For these reasons I have found it advisable to keep the animals separate and the cage shown in Fig. 11 which can be made by any carpenter, has been found satisfactory.

The tail blood of each animal is examined daily and the presence or absence of trypanosomes recorded. At least five fields are looked over before a negative result is recorded. If trypanosomes are present they are recorded by the *Few*, +, ++, +++ and ++++ scheme described above.

The untreated controls generally succumb in 5 to 7 days after infection. The survivors among treated animals are kept under observation for at least *three weeks;* trypanosomiasis may develop as late as 15 days after treatment which is 16 days after infection. The majority of animals remaining sterile for this period are cured but I extend the period to 21 days in order to make sure that the dose recorded as "curative" has effected complete sterilization.

A complete test should include controls receiving drug alone (but not infected) as checks upon the toxicity of the compounds being employed; as a general rule, however, toxicity tests are first conducted as described in Chapter II and under such conditions these controls may be omitted.

Of course it is not absolutely necessary to extend a test over a period of 21 days; it may be terminated at any arbitrary time but if this plan is adopted it should not be earlier than the 7th day at which time the controls will have succumbed. If such a plan is pursued the smallest dose of medicament preventing blood invasion is called the "*minimal effective dose*" (M.E.D.); the smallest amount preventing blood invasion for 21 days is designated as the "*minimal curative dose*" (M.C.D.) and rats succumbing after this time are not destroyed by trypanosomiasis but by late toxic effects of the drug injected or some other factor.

Advantages and Disadvantages of the Method; Uniformity of Results.—The advantages of the method are its accuracy, technical simplicity and economy of animals and labor. Ordinarily the doses given to rats vary by 1 milligram per kilogram of body weight but with some substances and particularly arsphenamin and neoarsphenamin, differences of ½ milligram may show appreciable effects upon the infections. With new compounds of unknown trypanocidal activity the usual procedure is to first determine the toxicity followed by a preliminary trypanocidal test in which the range of doses is higher as, for example, 0.001, 0.002, 0.004, 0.008, 0.010, 0.020 gm., etc., per kilogram of rat; when some idea of trypanocidal activity is thereby gained a re-test with closer doses employing 4 rats is done for more accurate data. If it is inconvenient to wait 21 days for the "minimal curative dose" the results may be read at the end of 5 to 7 days as the "minimal effective dose."

Ordinarily, therefore, 6 rats suffice for a preliminary test and 4 rats for a final test. After some experience with the method has been gained, an operator and assistant can easily infect and administer compounds to 30 rats within a total of four hours of working time; this permits the testing of many compounds with a minimum of labor and animals.

Arsphenamin being more stable than neoarsphenamin and much less subject to variations in toxicity and trypanocidal activity when properly preserved in vacuum tubes, has yielded fairly uniform results in trypanocidal tests at intervals over a period as long as seven months. In one of my experiments twelve different tests were conducted with the same lot of arsphenamin; in three of these tests the minimal curative dose was 0.004 gm. per kilogram; in eight tests the dose was 0.003 gm. and in one test 0.002 gm. In the majority of tests therefore, the dose was identical (8 out 12 tests) and the greatest variation over the period of seven months was but 0.002 gm. per kilogram (0.002–0.004 gm.).

A possible disadvantage is that treatment is given before blood invasion has become well advanced. This, however, is not a matter of much importance because it influences only the dose required for sterilization. As shown in the preceding chapter, the amount of trypanocidal substance required for sterilization when given immediately or within four hours after intraperitoneal inoculation, is generally higher than required after an elapse of 24 hours because the drug may be eliminated too rapidly. Likewise the amount required for sterilization after an elapse of 48 to 72 hours and longer is greater than required at the end of 24 hours because more trypanosomes require destruction. For these reasons at least 18 to 24 hours should elapse between infection and treatment but there is no advantage in waiting longer because the results become more irregular. After all the rat test is of value only for determining comparative trypanocidal activities; for results applicable to the actual treatment of human trypanosomiasis or infections of horses (as dourine) and other animals, additional tests employing rats or rabbits are advisable.

With a strain of *Tr. equiperdum* of uniform virulence and with rats of approximately the same age and weight infected with approximately the same number of trypanosomes, all of which are factors easily under control, the results are of acceptable uniformity. Under these conditions the same lot of arsphenamin preserved in vacuum has yielded a minimal curative dose of 0.004 to 0.006 gm. per kilogram of weight when tested at intervals over a period of one month; arsphenamin of manufacture by a second laboratory showed the same trypanocidal dose of 0.004 gm. in all four tests while the product of a third laboratory showed a value of 0.006 gm. in each of three tests and 0.005 gm. in a fourth test. In similar experiments with neoarsphenamin prepared by different laboratories slightly greater variation was noted.

The Arsphenamin Coefficient.—Probably the greatest single factor of variation in conducting trypanocidal tests by this or any other method, is variation in resistance of the strain of trypanosomes being employed. This is not always due to variations in virulence because untreated controls may not die sooner or survive longer than the usual periods of 5 to 7 days. But yet in some unaccountable manner changes in resistance of the strain to arsphenamin and neoarsphenamin may occur. Naturally one first surmises when the resistance is increased, that by accident the seed rat had received an insufficiently sterilizing dose of either compound and the surviving trypanosomes have propogated a race of resistant organisms. But this is apparently not always the case and it is not possible in any biological test employing two living organisms, namely, host and trypanosomes, to secure results of mathematical accuracy.

For this reason I have always included arsphenamin in each lot of trypanocidal tests because the results with arsphenamin indicate how the whole series of tests should be evaluated. Arsphenamin was selected after it was found that the same lot could be kept over a period of many months with no appreciably change in toxicity or trypanocidal activity when properly preserved. It serves in trypanocidal tests as a "coefficient" for expressing the trypanocidal values of other compounds. In this manner it is analogous to the use of phenol in the Rideal-Walker and Hygienic laboratory germicidal tests in which results are compared with standardized phenol and expressed as the "phenol coefficient." Likewise it serves as a constant factor in these tests like standard toxin or antitoxin for the testing of diphtheria and tetanus antitoxins in guinea pigs. *Even if the results are changed by variation in the resistance of the trypanosomes the "arsphenamin coefficient" is not greatly altered.* If the same arsphenamin cannot be carried along from month to month, any good arsphenamin is next best because experience has shown that arsphenamin of different lots possesses fairly uniform trypanocidal activities under proper conditions. Neoarsphenamin, however, varies to a greater extent and is not suitable as a coefficient of trypanocidal activity.

Hygienic Laboratory Method for Conducting Trypanocidal Tests Employing Rats.—In this method which has been developed in the Hygienic laboratory by Voegtlin and Smith in coöperation with Crane, Wright and Connell,[5] treatment is not begun until each rat shows from 100,000 to 300,000 trypanosomes per cubic millimeter of blood, the purpose being to determine the smallest amount of drug capable of killing these numbers. The trypanosomes of each animal must be counted and for this reason the method consumes a good deal of time. The technic is as follows:

Healthy albino rats from one breeding strain and weighing about 50 to 60 grams are put on a diet of milk bread and oats until they reach a weight of

100 to 150 grams, when they are ready to be used in this test. The animals should not be pregnant.

A strain of *Tr. equiperdum* is employed, being propagated in rats. A seed rat showing about 200,000 parasites per cubic millimeter of blood is bled without special asceptic precautions into 5 c.c. of saline solution containing two per cent. of sodium citrate. This suspension is then injected intraperitoneally into rats, using 0.5 c.c. per animal. Twenty-four hours later the rats so injected show 50,000 to 100,000 trypanosomes per cubic millimeter of blood, and die as a rule within two or three days if left untreated. For the tests rats are chosen which show 100,000 to 300,000 trypanosomes per cubic millimeter of blood; they have found that approximately 150,000 give best results. After the rats have been infected it is necessary next day to examine them for trypanosomes, first by the simple microscopical examination of the blood. If trypanosomes are present a count is made; if the number is less than 100,000 treatment is delayed for a few hours. If more than 300,000 are present the rat is not employed. The counts are made by my method previously described.

The drugs are administered by intravenous injection and the time of disappearance of the parasites from the blood stream determined by subsequent examinations and counts. At least two counts are made on the day of injection and a count on the following and second days.

The injection of the drug to be tested is carried out as follows: the drug is dissolved according to the usual procedure in distilled water avoiding all unnecessary shaking and adjusting the concentration in such a way that the desired dose is within 0.3 to 0.9 c.c. An accurately calibrated tuberculin syringe provided with a 26 gauge Luer needle, is then filled with the freshly prepared solution and the drug is immediately injected at a slow rate into the leg vein previously exposed by skin incision. It is not necessary to use anesthesia for this very simple operation. The rat is tied to a board provided with four nails to which the four legs are fastened by means of strings. The number of trypanosomes per cubic millimeter of blood should be within 100,000 to 250,000 preferably between 100,000 to 150,000.

Minimum Effective Dose.—In order to compare the trypanocidal efficienty of various lots of arsphenamin or neoarsphenamin graded doses of the drug are injected into a series of animals. It has been found that a variation of about 50 per cent. between successive doses (1, 1.5, 2, 25, 3, 75, 5, 7.5, 10, etc.) is all that can be expected of the accuracy of the test. This is due to the fact that quantitative differences in the metabolism of the drug and rate of excretion of the arsenic in different rats are sufficiently great to produce considerable variations in parasiticidal action. These variations make it necessary to use at least five rats for each dose. At the end of 24 hours after the injection of the drug the number of parasites in the tail blood is again determined, first by a preliminary examination of a drop for the presence or absence of parasites and then a count of the blood specimen which were found positive in the preliminary examination. The method permits to count with accuracy 1000 or more parasites per cubic millimeter. Below 1000 the count is unreliable and therefore they have refrained from making counts in such cases and merely go by the result of the drop examination, calling it a *trace*, when only one parasite is found in several different microscopic fields of a fairly thick preparation.

The minimum effective dose is the dose required to bring the parasitic count within 24 hours to a trace or negative, or in other words the dose which kills from 100,000 to 250,000 parasites per cubic millimeter of blood. As a

rule the blood is again examined at the end of 48 hours and 72 hours after treatment.

Extensive experience has shown that with arsphenamin and neoarsphenamin the maximum effect upon the parasites is exerted within the first 24 hours, though occasionally a count of 1000 or less may become trace or negative in 48 or 72 hours.

English Method for Conducting Trypanocidal Tests Employing Mice.—The method employed by the Medical Research Council of England for testing the trypanocidal activity of salvarsan and neosalvarsan and devised by Dale and White in coöperation with Burn, Durham and Marchal,[6] is essentially similar to the Hygienic Laboratory method of Voegtlin and Smith except that mice are used.

Tr. rhodesiense and *Tr. equiperdum* have been employed with almost identical results but the latter is now used routinely; comparative tests employing rats and mice yielded closely similar results but the latter has been adopted for routine tests because the toxicity tests are conducted with mice.

The strain of *Tr. equiperdum* is propogated in rats and mice are infected for the tests by intraperitoneal injections of 0.1 c.c. of an emulsion of rat's blood in 1 per cent. citrate saline solution so diluted that the total number of trypanosomes injected is in the neighborhood of 7–8 millions. Two days later mice are selected from the infected group showing 100,000 to 500,000 trypanosomes per c.mm. of blood; it is stated that about two thirds of the number of inoculated mice are generally ready for treatment at this time. The counts are made in the usual manner with blood from the tail diluted with Toison fluid to which a little formalin has been added.

The medicament is prepared in solution and administered by injection into one of the tail veins. The effects are observed by microscopic examination for the presence of trypanosomes in drops of blood taken from the tail and examined fresh 24, 48, and 72 hours after the injection of the drug. The least dose which causes, within 72 hours, a complete disappearance of trypanosomes in the sense that none can be found in a systematic search of the tail blood, is termed the "minimal curative dose."

Author's Method for Conducting Trypanocidal Tests Employing Guinea-pigs and Rabbits.—As previously stated trypanocidal tests employing guinea pigs and rabbits are not required for routine work; these tests are employed only in the special study of medicinal compounds for determining their curative activity in chronic trypanosome infections with pathological changes in the fixed tissues. They are especially desirable, however, for chemotherapeutic studies in the treatment of trypanosomiasis of human beings, as African "sleeping sickness," and in trypanosomiasis of the lower animals, as dourine of horses. They are also applicable in chemotherapeutic studies in the treatment of chronic syphilis of human beings, and with such compounds as arsphenamin and neoarsphenamin in which the measure of trypanocidal activity is acceptable as a measure of spirocheticidal activity. Either *Tr. equiperdum* or *Tr. brucei* may be employed propogated in rats.

Guinea-pigs of approximately 300 grams and rabbits of 1500 to 2000 grams are preferred. Both animals are infected by the intraperitoneal injection of approximately 5 million trypanosomes; there is much less influence upon the results of trypanocidal tests by the numerical strength of the infection than observed in rat tests. It is not absolutely necessary to count the trypanosomes if a ++++ seed rat is selected and bled to death in 10 c.c. of warm citrate saline solution; of this emulsion guinea pigs receive 1 c.c. and rabbits 2 c.c. intraperitoneally.

It is scarcely necessary to state that the results of trypanocidal tests are influenced by the length of interval between injection and treatment. If the medicament is given 24 hours after inoculation the trypanocidal dose is less than if given several days or a week after inoculation; for example, the trypanocidal dose of arsphenamin given intravenously 24 hours after the intraperitoneal injection of guinea-pigs was approximately 0.006 gm. per kilogram of weight; when one week was allowed to elapse before the drug was administered the trypanocidal dose was approximately 0.015 gm. per kilo.

Since the special purpose of the test is to determine the influence of the medicament in the presence of tissue lesions, I allow 7 *days to elapse after inoculation before treatment is begun.*

The medicaments are given intravenously. Each animal is carefully weighed after fasting at least 12 hours and pregnant animals are avoided. The dose is made up separately for each animal so that a given amount is administered per kilogram of body weight in 2 c.c. for guinea-pigs and 5 c.c. for rabbits. In each experiment controls are included consisting of at least 2 infected animals without treatment. Drug controls are not ordinarily required if the doses given are known to be less than the toxic amounts for guinea-pigs and rabbits.

Guinea-pigs are injected by means of a saphenous or external jugular vein, the technic being essentially the same as described for the injection of rats. Rabbits are injected by means of an ear vein.

The animals are now kept under observation for at least 4 to 6 weeks, the blood (obtained from an ear vein of either guinea pig or rabbit) being examined at frequent intervals (usually every other day) and an additional record made of the development of any of the skin, mucous membrane and genital lesions. At the end of this time the spleen of each animal is removed and ground up in about 5 c.c. of sterile saline solution; 1 c.c. of the suspension is then injected intraperitoneally into each of two rats and these are observed for 6 or 7 days for the development of trypanosomiasis. This control test is required before one may be certain that an animal is cured, being anologous in this respect to the removal of lymph-glands and injection into fresh rabbits when conducting chemotherapeutic tests with syphilitic rabbits (see Chapter XXI). Sometimes guinea-pigs and rabbits at the end of 4 to 6 weeks are apparently in good condition and free of blood and tissue trypanosomiasis until the rat test discloses that living trypanosomes are present in the spleen. Emulsions of liver may be used instead of the spleen.

Author's Methods for Determining the Trypanocidal Activity of Substances in Vitro.—During the course of chemotherapeutic studies, compounds possessing a well-defined parasitropic effect may be prepared which prove so highly organotropic or toxic for the test animal that the minute doses tolerated fail to exert an appreciable influence on the parasites. The mercurials, which are known to exert a powerful bactericidal effect *in vitro*, are substances of this class, being so highly toxic that doses greater than 0.0004 gm. per 100 gm. of white rat are apt to prove fatal and yet are too small materially to influence the course of experimental trypanosomiasis.

In order, therefore, to avoid the mistake of discarding new compounds possessing a high degree of trypanocidal activity because they are highly toxic for experimental animals, a study of various methods of determining the trypanocidal activity of a drug, independent to some extent of its toxicity for the body cells of the host, was undertaken. In the event of the preparation of a new compound that is possessed of a high degree of parasitropism, but is also highly toxic, the efforts of the experimental chemotherapeutist are devoted to lowering the toxicity to a sufficient extent to permit of the administration of the drug to living animals.

With the purpose, then, of devising a technic for determining the parasiticidal activity of a chemical independent of its toxicity for body cells, I studied various methods of determining the trypanocidal activity of substances *in vitro*, which have been published in coöperation with Schamberg and Raiziss[7]. There is considerable evidence at hand that substances exhibiting a marked parasiticidal effect *in vitro* are likely to act in a similar manner *in vivo*, provided the toxicity is sufficiently low to permit of the administration of fair-sized doses; this is true to a certain extent of arsphenamin and neoarsphenamin, likewise of ethylhydrocuprein hydrochlorid and other quinin derivatives in the treatment of experimental pneumococcus infections as shown by Steinfield and myself.

Since the cultivation of pathogenic trypanosomes, as *Tr. equiperdum* and *Tr. brucei*, on artificial media yields irregular results, these methods were designed to determine the influence of a drug upon trypanosomes *in vitro*

by loss of motility and structural changes as determined by direct microscopic examination, and more particularly whether or not the trypanosomes were killed as determined by injecting them into rats after they have been exposed to the medicament in test tubes.

Technic of Microscopic Method.—A series of dilutions of the chemical in 0.85 per cent. sodium chlorid or Ringer's solution and twice as strong as those desired for action on the trypanosomes, is prepared in amounts of 0.5 c.c. and placed in small sterile test tubes in a suitable rack in a pan of water at 37°–40°C.

A seed rat carrying the trypanosomes for the tests is selected whose blood, as examined microscopically with a ⅙ objective and No. 4 eyepiece (Leitz) by placing a drop from the tail upon a cover glass and suspending it over a concave slide, shows a large number of active trypanosomes. One cubic centimeter of a 1 per cent. solution of sodium citrate in normal salt or Ringer's solution is placed in a test tube and warmed; sufficient blood is then secured from the heart or tail of the seed rat to give an emulsion of trypanosomes which, when examined as described above, will show at least 10 trypanosomes in each field and particularly at the margin of the drop. The sodium citrate is added to prevent coagulation of the blood, but more than a 2 per cent. solution is not advisable. This blood trypanosome emulsion is kept warm by placing the tube in the water-bath.

Slides having two or more concavities are prepared and ringer with vaselin. With a 3-mm. platinum loop a drop of a dilution of the chemical is placed in the center of a cover glass; the wire is heated and cooled and a drop of the blood emulsion added and mixed in exactly the same manner as in preparing a microscopic agglutination test. The cover glass is then adjusted to a concavity in the slide and carefully sealed with vaselin. The dilution of chemical now acting upon the trypanosomes is doubled. The time when blood and chemical are mixed is carefully noted, and the slide placed on a warm stage of the microscope or in a small oven heated to 37°–40°C. placed alongside of the microscope.

A number of preparations are made as rapidly as possible with the aid of an assistant, including at least 2 controls prepared by mixing a drop of trypanosome emulsion with a drop of warm normal salt or Ringer's solution.

It is advisable to keep the solutions of chemical, the trypanosome emulsions, and the finished slides at body temperature in order to avoid the effect of chilling on the trypanosomes.

At definite intervals, ranging, for example, from 5 to 10 minutes to an hour or longer from the time of exposure of the trypanosomes to the chemical the slides are examined microscopically and compared with the controls.

As a general rule, the controls show active trypanosomes for at least 1 to 2 hours or longer; after this time the movements may become sluggish or cease entirely in a few of the parasites. For the purpose of avoiding error it is my custom to terminate the experiment at the end of 1 hour.

In case the chemical under study exerts an influence on the trypanosomes the parasites first lose their to and fro movements and remain in one position with constant vibratile movement. Later the latter movement becomes more and more sluggish, finally ceasing altogether, and certain structural changes occur. These effects are best determined by inspecting the trypanosomes at the margin of the drop; before motility is regarded as completely destroyed, a number of fields should be carefully inspected.

With practice and assistance a large number of preparations may be made in a short time. It is our custom to permit at least one-half minute for the preparation of each cover glass. As a general rule it is advisable to examine a preparation within 10 minutes of the time when chemical and trypanosomes are mixed; strong dilutions of a trypanocidal substance, as arsenobenzol, produce an effect with a few minutes.

This method is open, however, to some degree of error. The results of experiments with the same substance at different times with the same strain of trypanosomes have been found to vary; hence the results should be interpreted as showing approximately the relative trypanocidal activity of different substances judged solely by their influence on the motility and appearance of the parasites. Loss of motility, however, is not necessarily an indication of the death of a parasite.

Technic of Combined in Vitro-in Vivo Method.—In this method equal parts of varying dilutions of the chemical under study are mixed with blood trypanosome emulsion and kept at 37°–40°C., when the whole or a part of each is injected intraperitoneally into white rats to determine the degree of trypanocidal activity. As a part of the drug is injected, the action of the drug is both *in vitro* and *in vivo* and due care must be exercised against the administration of lethal doses of the drug.

A series of dilutions of the drug under study is prepared in sterile normal salt or Ringer's solution in small sterile test tubes (1 × 6 cm.) and in amounts of 0.5 c.c. The series is placed in a water-bath at 37°–40°C. to be warmed to body temperature.

A rat heavily infected with *Tr. equiperdum* or *Tr. brucei* is selected, and a count made of the number of trypanosomes per cubic centimeter of blood.

Blood is then secured from the heart or vessels of the neck in sufficient sterile 1 per cent. solution of sodium citrate in normal salt or Ringer's solution warmed to 40°C. to give the desired dilution. Each cubic centimeter of this suspension should contain at least 20,000,000 trypanosomes. After a count has been made and the amount of dilution calculated, it is good practice to place the diluent in a small graduated cylinder and bleed the rat from the neck into the cylinder by means of a sterile funnel until the desired amount of blood has been added. This mixture is then gently shaken and afterward kept at a temperature of 37°–40°C. in a water-bath.

At an appointed time 0.5 c.c. of the trypanosome emulsion is added to each tube containing the solution of chemical and to a series of controls containing 0.5 c.c. of sterile normal salt or Ringer's solution. Each tube is gently shaken and then kept in the water-bath at 37°–40°C. for 15 minutes. The trypanosomes should be added to the chemical at intervals of one-half minute, which permits sufficient time for the injection of each.

At the end of the period of exposure 0.5 c.c. of the blood trypanosome chemical mixture is injected into the peritoneum of a rat. One-half minute is assigned for each injection. A 1-c.c. record syringe fitted with a 22 platinum iridium needle is well adapted for the injections. After each injection there is sufficient time to wash out the syringe and needle several times with hot normal salt solution conveniently placed near the operator.

Each rat is then kept under observation for at least 2 weeks and the blood examined daily for trypanosomes. In this manner the degree of trypanocidal activity may be determined according to the time when trypanosomes appear and the duration of life as compared with that of the controls. As a part of the chemical is injected into the peritoneum of the rat, its trypanocidal activity is probably continued *in vivo*.

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CHAPTER XI

TOXICITY AND TRYPANOCIDAL ACTIVITY OF DYES

As stated in Chapter V the anilin dyes were among the first substances to command interest in the chemotherapy of experimental infections and especially in the treatment of trypanosomiasis of the mouse and rat. While several were found to possess a high degree of trypanocidal activity none have proven of any particular practical value in the treatment of trypanosome diseases of man and the lower animals, although the early investigations in chemotherapy of experimental trypanosomiasis with dyes proved a wonderful stimulus and aided greatly in establishing some of the fundamental principles of this new field in therapeutic science. Work of this kind necessarily demanded the trial of hundreds of new compounds, and the comparative technical ease of trypanocidal tests and the relatively short time required for the evaluation of results, rendered them of much value.

I have already classified the more important anilin dyes in relation to chemotherapy in Chapter V. Not all have been tried out in trypanosomiasis, only some belonging to the azo and triphenylmethane series receiving much attention. It cannot be too strongly emphasized however, that the method employed for the conduct of trypanocidal tests has a marked influence upon

the results. The organism employed, the test animal, the number of organisms used for producing infection and especially the interval between infection and treatment, are important modifying factors. Therefore, the results recorded in the succeeding pages of this chapter and in Chapters XII to XV should be broadly interpreted. All values expressed by Schamberg, Raiziss and myself or by Voegtlin and Smith are based upon the two methods described in the preceding chapter; European investigators, however, have not always described their methods, but in general terms the tests have been usually conducted with mice by a technic similar to that of mine employing rats.

Toxicity and Trypanocidal Activity of Azo Dyes.—Owing to Ehrlich's interest and pioneer investigations in the staining of cells and particularly the subject of vital staining, studies in the trypanocidal activity of certain dyes early claimed his attention. With the cooperation of Shiga a large number of dye compounds were tested and encouraging results observed with some belonging to the azo group of the benzidine series, especially the *benzopupurin* series. Ehrlich had noted that these dyes remained in the blood for variable periods of time and still longer as granular deposits in certain cells of the internal organs, and these observations indicated a possible therapeutic application. Later Bouffard[1] showed that *trypan-blue* persisted as granules in the epithelium of the collecting tubules of the kidney, the Kupfer cells of the liver and in the connective tissue of numerous organs, the nervous system remaining conspicuously free. Decoloration and elimination of the dye first occurred in the plasma and then the cells. It was work of this kind on vital staining, therefore, that played an important role in early chemotherapeutic investigations.

At this time Ehrlich succeeded in enlisting the aid of Weinberg, an expert in the manufacturing of dyes, and with his assistance the solubility and trypanocidal activity of dyes of the benzidine series was increased by the introduction of sulphuric acid groups resulting in the discovery of a new compound designated as *trypan-red*.

Ehrlich and Shiga[2] found that this dye possessed a low toxicity for mice, the lethal dose by subcutaneous injection being about 0.5 gm. per kilogram of weight. When the compound was administered to mice by subcutaneous injection in doses of 0.05 to 0.5 c.c. of 1 per cent. solution per 20 grams of weight at the same time as the animals were infected with *Tr. equinum*, the mice were not taken ill and survived indefinitely. When treated three days after infection, the majority survived, although relapses occurred which could be controlled or avoided by subsequent injections. Ehrlich and Shiga also found that if mice were fed for two to eight days with cakes containing 0.2 gm. trypan-red that they could not be successfully infected with trypanosomes. With *Tr. brucei*, however, the results were negative both after feeding and subcutaneous injection of the compound, indicating the elective trypanocidal effects of this substance; furthermore, as shown subsequently, trypan-red is ineffective or almost so in *T. equinum* infections of the guinea pig, rat and dog although quite effective for the treatment of mice infected with this trypanosome. Curiously, trypan-red does not readily kill trypanosomes *in vitro* and does not stain the organisms until they are dead. Trypanosomes exposed to the dye *in vitro* however, have their virulence reduced even though they may not be killed.

According to their experiments trypan-red is not trypanocidal in the test tube. For this reason they advanced the theory that the dye engendered or greatly aided in the production of antibodies which constituted the chief trypanocidal agents. Ehrlich[3] later reported that 50 substitution com-

pounds of trypan-red were prepared and tested employing *Tr. brucei*; in all of those possessing some degree of trypanocidal activity the sulphuric acid rests were located in the naphthalene nucleus in the position 3.6 while the newly introduced groups were best placed in the position 7.

In 1906 Nicolle and Mesnil[4] published the results of their extensive studies with the dyes of the benzidine group, including *toluidin* and *trypan-blue*. The toxicity was determined by subcutaneous injection in mice and therapeutic activity by administration to mice infected with different strains of pathogenic trypanosomes, as *Tr. brucei*, *Tr. evansi* and *Tr. equinum*. Their work was especially notable by reason of the fact that they clearly showed the relation between chemical constitution and biological effects, the basis of chemotherapy and the goal of Ehrlich's efforts. For example, it was shown with this group of dyes that for trypanocidal activity the napthalene chains should contain the NH_2 group and at least two SO_3H groups. Dyes without these groups as dioxynapthalene disulphat, chlorooxynaphthalen disulphat and amidonaphthol disulphate usually did not stain the mice and were without trypanocidal activity. On the contrary the naphthylanimes, amidonaphthols, naphthalene diamines disulphat, the trisulphate combined with benzidine bases (toluidin, toluilene diamin monosulphate, etc.) usually stained the mice and proved trypanocidal in varying degree. Moore, Nierenstein and Todd[5] likewise found that the NH group in the aniline dyes was the active trypanocidal or "trypanophobe" group. Of these dyes belonging to the benzidine series trypan-red and trypan-blue have commanded most attention and especially the former. Trypan-blue in common with trypan-red, has a sulphuric acid radical adhering to the naphthalene ring occupying the position 3.6; as shown by Ehrlich and von Weinberg all the effective benzidine dyes show this position of the sulphuric acid radical.

The first indication of the importance of the ortho position was furnished by these studies with trypan-red and trypan-blue and their homologues, in which the azo derivative in the ortho position enters into the amido group of naphthalin.

Both of these dyes possess feeble trypanocidal activity; as shown by Vassal[6] trypan-blue is also effective against *Sp. recurrentis* in rats.

From the practical standpoint, however, trypan-red and trypan-blue have not proven of value in the treatment of trypanosomiasis of human beings or the lower animals, the arsenicals having proved more effective.

Toxicity and Trypanocidal Activity of the Triphenylmethane and Other Dyes.—These dyes were first studied by Wendelstadt and Fellmer.[7] Malachite green has proven too toxic and a reduction in toxicity by the introduction of a sulphuric acid molecule also results in a reduction of the trypanocidal effects. Ehrlich has observed that this was true of a series of sulpho acids of the basic dyes. The introduction of a carbonic acid group results in a reduction of trypanocidal activity, as in the case of *chrome-blue*, *chrome-violet* and *azo green;* likewise if a dydroxyl group is introduced, toxicity is reduced but likewise trypanocidal activity as in the case of tri-meth-oxy-para-rosanilin and tri-chlor-para-rosanilin.

Brilliant green was effective against experimental *Tr. brucei* infections in rats and monkeys when administered in repeated doses; negative results were observed with a dog. Microscopical examination of the blood during treatment showed morphological changes in the trypanocomes brought about by the dye. Similar results were observed by Weber and Krause.[8]

Ehrlich also found that derivatives of rosanilin as *hexamethyl-violet* and *hexaethyl-violet* possessed some trypanocidal activity in mice but all of these

showed a tendency for the production of extensive necrosis when injected subcutaneously.

A distinct advance was made with the discovery of *parafuchsin* by Ehrlich and more especially a halogenized derivative of this dye designated as "*tryparasan*" and discovered by Bender, in which chlorine enters in ortho position to the NH_2 group. Tryparasan has been studied by Roehl[9] who found that while mice do not bear more than 1 c.c. of 1:1000 to 1:1200 dilutions of parafuchsin per 20 grams of weight, tryparasan is well borne in dilution of 1:500. One injection of parafuchsin in the maximum tolerated dose delays trypanosomiasis in mice infected with *Tr. brucei* for 14 to 17 days but does not cure unless repeated doses are given. One injection of tryparasan, however, may bring about a complete cure. Both parafuchsin and tryparasan, however, may bring about a complete cure. Both parafuchsin and tryparasan are well borne when fed to mice and favorably influence trypanosome infections by this route of administration; 1 gram is dissolved in 90 c.c. of alcohol and 10 c.c. of oleic acid. Cakes are saturated with 1 c.c. of this mixture carrying 0.010 gm. of the dye. Marks[10] also found that feeding the drug favorably influenced trypanosome infections.

Weber and Krause[11] and Brieger and Krause[12] have studied the biological effects of some dyes belonging to the *acridin, safranin* and *euhordine* series. Especially favorable results were observed with *trypasafrol* of the safranin series, when fed to rats and guinea pigs within twenty-four hours of the time of infection with *Tr. brucei.* Dyes of the safrine series containing a combination of trivalent and pentavalent N were generally non toxic and effective in feeding experiments. The substitution of methyl groups for the H atoms in the animo groups usually resulted in a loss of trypanocidal activity and an increase of toxicity. When an H atom of the amido group was replaced by an acetic acid group, trypanocidal activity was likewise diminished and toxicity increased.

Toxicity and Trypanocidal Activity of "Bayer 205."—Recently a new compound designated as "Bayer 205" has been found to possess well marked curative activity for experimental trypanosomiasis. The exact composition of the substance is not known. The compound has been reported as successful in the treatment of trypanosomiasis of human beings and the lower animals and I shall refer to it again in Chapter XX.

Strictly speaking this substance, discovered by Handel and Joetten in 1920, is not a dye but a colorless, water soluble salt of a complex sulphonic acid. It is apparently one of a long series of compounds formed of chains of aminobenzoyl radicles, united by amid linkages, with a central urea linkage and terminal sulphonic acid groupings. The exact composition is a trade secret but it would appear that Fournier's "309" is of similar composition and action.

"Bayer 205" rapidly cures mice, rats, rabbits and dogs infected with several different strains of pathogenic trypanosomes; infected horses and oxen are cured with difficulty or not at all. Mice infected with *Tr. lewisi* are usually not cured. It has little or no effect *in vitro* suggesting that its curative activity requires the cooperation of the tissues of the host.

One of the remarkable properties of this substance is the long persistence of its effects. While a mouse or rat may be cured in a few days yet they cannot be re-infected for weeks or months and during this time their serum or extracts of certain organs injected into other infected animals results in curative effects. The substance has a large molecule but is extremely soluble in water and readily diffusible through collodion membranes. It is difficult to understand therefore, why it persists in the cells. It has been suggested

that the compound is in the blood and cells in combination with proteins because after standing an hour or two in contact with serum, the compound no longer passes through collodion filters and if the proteins are coagulated by heat, it is not found in the filtrate.

According to Wenyon[13] the maximum tolerated dose for mice by subcutaneous injection is about 0.5 gm. per kilo and the minimum curative dose for *Tr. equiperdum* infections is about $\frac{1}{60}$th this amount. According to Haendel and Joetten[14] and Mayer and Zeiss[15] the compound is active against experimental infections produced by *Tr. brucei, Tr. equinum, Tr. rhodesiense* and *Tr. gambiense,* but not for *Tr. lewisi* and *Tr. cruzi.* Hesselbach[16] found that $7\frac{1}{2}$ per cent. solutions destroy trypanosomes *in vitro* in 25 to 60 minutes. According to Mayer and Menk[17] the compound may be found in the blood (in union with the serum proteins) thirteen days after injection and in the urine twelve days. After the administration of 2 grams by mouth it was found in the serum for eight days.

Mechanism of Trypanocidal Activity of Dye Compounds.—The exact mechanism of the trypanocidal activity of various dye compounds is unknown. Ehrlich and Shiga observed early in their work that solutions of trypan-red possessed but slight trypanocidal activity in the test tube and Ehrlich assumed that its trypanocidal activity in the body was due to the production of new compounds analogous to the formation of the trypanocidally active "oxid" compounds from the arsenicals of the arsenobenzol group by processes of oxidation in the body. In regard to the benzidin dyes Ehrlich has assumed that their activity may be directed toward preventing the multiplication of trypanosomes since Busck showed that free living parasites in solutions of trypan-red may be prevented from dividing and multiplying even though motility and life are not influenced. Nierenstein has expressed the opinion that the amino group of such dyes as trypan-red, Afridol-blue, Afridol-violet and parafuchsin, is directly trypanocidal.

Influence of Dyes upon the Immunity Following Recovery in Experimental Trypanosomiasis.—In 1904, Ehrlich and Shiga[18] discovered a new mechanism having a bearing upon the action of trypan-red in trypanosomiasis of mice. It was observed that mice infected with *Tr. equinum* and cured with one or more injections of trypan-red possessed an immunity to re-infection lasting eighteen to twenty days. In other words, as a result of the cure a temporary immunity had developed, although no signs of immunity developed among untreated mice and none of the untreated animals ever recovered or even developed a chronic infection. Attempts to prolong the immunity by repeatedly infecting and curing mice in the course of a number of weeks, were not very successful. Subsequently, Ehrlich[19] studied the immunity which followed the cure of *Tr. brucei* infections, and found the immunity of shorter duration (about ten days). According to Ehrlich, the duration of the immunity depends upon two factors: (1) the height of the immunity reached, and (2) the virulence of the trypanosomes employed to overcome the immunity. The higher the virulence of the organisms, the shorter the immunity.

Ehrlich and Shiga showed that this immunity after cure with trypan-red was not due to unexcreted dye as is probably the case of "Bayer 205," since normal animals first treated with the dye could be infected although the incubation period may be prolonged.

Halberstaedter[20] then discovered that the immunity was specific. A mouse cured of *Tr. equinum* infection was susceptible to *Tr. equiperdum* and upon being cured of this infection was in turn susceptible to *Tr. brucei.* Ehrlich, however, was not of the opinion that this reaction would serve to differentiate among strains of trypanosomes belonging to different species because the reaction appeared to be too delicate.

At this stage the subject was very thoroughly studied by Terry[21] who employed three different strains of *Tr. evansi* and a single strain each of *Tr. equiperdum*, *Tr. equinum* and *Tr. brucei*. The therapeutic agents employed were amidonaphtol disulphonic acid 1.8.3.6 plus dichlorbenzidin, arsacetin, trypan-red, arsenophenylglycin and various combinations. Best results were observed by employing one of the dyes, either alone or in combination with arsacetin.

Terry was able to confirm the extreme delicacy of the immunity in that mice recovering under treatment from one strain of *Tr. evansi* were still susceptible to other strains of these trypanosomes and by means of this immunity reaction it was apparently possible in a number of instances to separate in purity organisms that had been mixed *in vitro*.

Immunity was demonstrated with all strains and appeared to be a general phenomenon. As a rule it developed early, being detected at times between the second and third days after treatment. The immunity, however, was always temporary and rarely lasted beyond twenty days. Best immunity followed the cure of *Tr. evansi* infections and in general terms was better after the cure of virulent infections than after infection with less virulent trypanosomes.

Influence of Dyes upon Antibody Production in Trypanosomiasis.—The mechanism of this peculiar immunity following recovery from trypanosomiasis under treatment with dyes alone or in combination with arsenical compounds, has never been thoroughly determined. Apparently the medicament either (1) lowers the virulence of the parasites and thereby affords the cells of the host a better opportunity of protecting themselves with the production of immune substances; or, (2) the medicament causes the more rapid release of antigenic substances from the parasites; or, (3) the medicament stimulates the body sells to a more rapid and efficient production of antibodies.

Whether the immune substances are present in the blood of the recovered animals or remain in or upon the body cells has not yet been satisfactorily determined, although some experiments have shown that the serum is trypanocidal. Franke[22] for example, has shown that the serum of a monkey cured of *Tr. equinum* infection, was definitely trypanocidal and that the trypanosomes may develop a resistance or "fastness" to these serum antibodies. I have also found trypanocidal substances in the sera of animals recovering from *Tr. equiperdum* infections as the result of treatment with arsphenamin and neoarsphenamin.

Acquired Resistance of Trypanosomes to Dyes.—I have already discussed in Chapter I this interesting phenomenon which bears a very important relation to the chemotherapy of disease. Franke and Rohl working in Ehrlich's laboratory, discovered that the reason why mice infected with *Tr. brucei* and treated with parafuchsin could ultimately succumb to a recurrence of the infection in spite of treatment with more parafuchsin, was because the organisms had acquired a "resistance" or "fastness" against the effects of parafuchsin. When these trypanosomes were injected into fresh mice the infection was from the very beginning uninfluenced by parafuchsin, administered either by feeding or by injection. Rohl so increased the "resistance" of this strain that on inoculation into animals fed with parafuchsin for days or even weeks beforehand, the infection proceeded as in normal mice, whereas mice so treated and inoculated with a normal strain were protected against infection in 90 per cent. of cases.

Browning[23] succeeded in creating a strain of *Tr. brucei* resistant to trypan-blue in four weeks and resistance to trypan-red in two weeks.

A strain rendered resistant to parafuchsin was also resistant to Dobners violet. A trypan-blue resistant strain was found resistant to trypan-violet and to trypan-red, and the trypan-red resistant strain was resistant to trypan-blue. This group resistance indicates therefore, that the fastness is not absolutely specific but is present in more or less degree for substances of the same chemical type. In the case of these benzidin dyes the phenomenon is of much interest inasmuch as the only feature in common to all is the presence of sulphonic radicals (HSO_3) in the position 3, 6 in the naphthalin-nuclei of the side chains.

However, when the chemical compounds are unrelated, group resistance is not found. Strains of trypanosomes resistant to the dyes as parafuchsin, trypan-red or trypan-blue are just as susceptible to atoxyl or other arsenical compounds as normal strains; a single strain may, however, be rendered resistant to a dye and an arsenical as previously mentioned and when such a strain is inoculated into mice, or, when mice are infected with two different resistant strains, the two varieties behave as if they were distinct species, even though derived from the same original strain. Browning and Gulbranson[24] recently showed that mice protected against infection with virulent *Tr. brucei* by feeding them with parafuchsin before inoculation, could be infected by inoculating them with a parafuchsin-resistant strain. In other words parafuchsin stored in the tissues was trypanocidal for a normal strain, but ineffective against a resistant strain.

Once acquired the resistance to dyes persists for long periods of time. Browning records that a trypan-blue-resistant strain was still highly resistant after eighty passages extending over six months. So also, after five passages through rabbits over a period of five months, the high specific resistance to trypan-blue was still retained.

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CHAPTER XII

TOXICITY AND TRYPANOCIDAL ACTIVITY OF ARSENICAL COMPOUNDS

Trypanocidal tests with the arsenical compounds have long been recognized as possessing particular and practical value, not only in relation to chemotherapeutic investigation but especially in relation to the development of compounds for the treatment of syphilis, since it has been found that with these compounds there is relationship between trypanocidal and spirocheticidal properties.

Toxicity and Trypanocidal Activity of Inorganic Compounds of Arsenic. The inorganic compounds of arsenic possess but feeble trypanocidal activity even in doses approaching the maximum tolerated amounts. A single dose usually does not influence either the severity or duration of trypanosomiasis in rats infected with *Tr. brucei* or *Tr. equiperdum*, although repeated doses in sublethal amounts may prolong the lives of experimental animals.

Laveran and Mesnil[1] administered *arsenious acid* (lethal dose 0.015 to 0.020 gm. per kilo) to rats, mice and dogs infected with *Tr. brucei* or *Tr. equinum* and prolonged their lives but never effected a complete cure, the animals dying of poisoning if too much arsenic was administered and of trypanosomiasis if treatment was interrupted. Loeffler and Rühs[2] claim to have succeeded in curing guinea-pigs, rabbits and rats infected with *Tr. brucei* by administering arsenious acid intraperitoneally in 3 to 5 doses of 0.005 to 0.0075 gm. per kilogram. In the experiments of Schamberg, Raiziss and myself, conducted with rats infected with *Tr. equiperdum*, the administration of arsenious acid only prolonged the lives of animals for a few days but never effected a complete cure; *sodium arsenite* (maximum tolerated dose 0.003 to 0.005 gm. per kilogram), in which arsenic is present in trivalent form and *sodium arsenate* (maximum tolerated dose 0.040 to 0.050 gm. per kilogram), in which arsenic is present in pentavalent form, never proved effective in single large doses although in repeated smaller doses the lives of the animals were sometimes prolonged by several days.

The inorganic compounds of arsenic, therefore, including both the highly toxic trivalent compounds of arsenious acid and the less toxic pentavalent compounds of arsenic acid, possess but slight or no demonstrable trypanocidal activity in acute fulminating infections as in mice and rats infected with *Tr. brucei*, *Tr. equinum* or *Tr. equiperdum;* in chronic tissue infections in guinea-pigs and rabbits with these trypanosomes, it is possible to demonstrate slight degrees of trypanocidal activity by administering repeated doses in amounts approaching the maximum tolerated doses. Indeed, Laveran and Mesnil had been influenced to undertake a study of the trypanocidal activity of the arsenicals by the favorable reports of Lingard[3] and Bruce[4] on the treatment of surra and nagana with arsenic.

Toxicity and Trypanocidal Activity of Organic Compounds of Arsenic; *Atoxyl.*—The results of toxicity and trypanocidal tests with *atoxyl* or sodium arsinalate (sodium salt of 4-aminophenyl arsonic acid) have varied greatly according to the chemical purity of different lots, the maximum tolerated dose for rats by intravenous injection being given as 0.05 to 0.150 gm. per

kilogram (general average about 0.150 gm.). Although discovered by Béchamp[5] in 1860 by combining anilin and arsenic acid and first regarded as a simple meta-arsenic acid anilid, it was not until 1907 that Ehrlich and Bertheim discovered that it was an amino derivative of phenylarsonic acid and opening up a large field for preparing new compounds by substitutions in the amino group.

Thomas,[6] in 1905, studied its toxicity and trypanocidal activity quite extensively in monkeys, dogs, rabbits, guinea-pigs, rats and mice infected with *Tr. gambiensi, Tr. evansi, Tr. brucei, Tr. equinum, Tr. equiperdum* and *Tr. dimorphon.* According to his experiments the drug had no appreciable effect upon the trypanosomiasis during the first three hours after injection, but after four to five hours the parasites appeared to be affected because their movements became less rapid with alterations in shape and size. After seven hours, their numbers in the peripheral blood were decreased with total disappearance after eighteen hours when a sufficiently large amount had been injected. Similar results were observed with trypan-red, only the trypanocidal effects were slower, requiring forty-eight hours; at this time Thomas suggested that a combination therapy of atoxyl and trypan-red may be efficacious in the treatment of trypanosome infections of man. Koch,[7] in 1907, tried atoxyl in the treatment of African sleeping sickness on a large scale and his first report inspired great hopes for success, but later it was found that relapses commonly occurred and that large doses may produce optic atrophy. I shall refer to this work again in the succeeding chapter. Uhlenhuth, Gross and Bickel,[8] in 1907, found that the administration of atoxyl in dose of 1 c.c. of a 1 per cent. solution to rats by subcutaneous infection on each of three successive days preceding infection with *Tr. equiperdum* did not prevent the development of a fatal trypanosomiasis. Better results were observed when a dose corresponding to about 0.2–0.3 gm. per kilogram of weight was given at the same time as the infection or smaller doses at intervals after infection. Similar results were observed by Marks, Weber and Kraus, Tsuzuchi, Voegtlin and Smith,[9] and Schamberg, Raiziss and myself. The total of these investigations have in general terms confirmed the work of Thomas, Uhlenhuth and their associates proving the trypanocidal activity of atoxyl in sublethal amounts for different pathogenic organisms in rabbits, rats and mice and for *Tr. equiperdum* infections of rats, the chemotherapeutic index being about 2.5, as shown in the following equation:

$$\frac{\text{Maximum tolerated dose: 0.150 gm. per kilogram}}{\text{Minimal curative dose: 0.06 gm. per kilogram}} = 2.5$$

Arsacetin, or sodium acetyl arsanilate, the sodium salt of 4-acetyl-aminophenyl arsonic acid, is a compound closely allied to atoxyl and was first prepared by Ehrlich and his associates.

The biological properties of this substance have been studied more extensively in relation to spirochetic infections and will be referred to in more detail in Chapter XXIII. Voegtlin and Smith have observed that it is considerably less toxic than atoxyl and more trypanocidal, possessing a chemotherapeutic index about five times higher than atoxyl. Browning[10] found that the maximum tolerated dose for mice by subcutaneous injection was about 3.0 grm. per kilogram of weight and that mice infected with *Tr. brucei* or *Tr. equiperdum* could be cured by the subcutaneous injection of 1.2 to 2.0 grm. per kilogram. Browning also observed practically the same toxicity and trypanocidal properties with a *para-oxy-benzylidene derivative of atoxyl.*

The cacodylates, aliphatic compounds containing arsenic in the pentavalent form, are close derivatives of arsenic acid. By substituting two methyl groups for two hydroxyl groups in arsenic acid, *cacodylic acid* is formed, and by further substitution of sodium for the hydrogen of the remaining hydroxyl group, *sodium cacodylate* is formed. Castelli,[11] Schamberg, Raiziss and myself,[12] Voegtlin and Smith[13] have found cacodylic acid and sodium cacodylate of low toxicity (0.2 gm. per kilogram), probably due to the fact that reduction in the body occurs slowly and a large percentage may be eliminated unchanged; however, trypanocidal activity is likewise almost absent and even doses as large as the maximum tolerated amounts have been found without appreciable effect in rats infected with *Tr. equiperdum* and *Tr. brucei*. Sodium cacodylate has been widely employed for the treatment of syphilis and doubtless does exert some degree of curative activity, but its use is not now justified in view of the fact that it has been demonstrated that its spirocheticidal activity, as well as its trypanocidal activity, is very slight and greatly superseded by arsphenamin and neoarsphenamin.

Sodium methyl arsenic acid or "arrhenal" is closely related to cacodylic acid, differing from sodium cacodylate in having one of its methyl groups replaced by an NaO group. This substance was introduced as a substitute for sodium cacodylate and at one time attracted considerable attention in the treatment of syphilis and human trypanosomiasis. It has been studied by Laveran and Mesnil, Castelli and Voegtlin and Smith; while somewhat less toxic than cacodylic acid, it has likewise been found ineffective in the treatment of trypanosomiasis of the rat and mouse even when administered in doses as high as the maximum tolerated amounts.

The same has been found true of another homologue of cacodylic acid, namely, *disodium ethyl arsonate* or *"mon arsone,"* which differs from "arrhenal" in having the methyl group replaced by an ethyl group. I have found that the maximum tolerated dose of this substance for white rats by intravenous injection is about 0.200 gm. per kilogram of body weight, but doses as high as 0.150 gm. per kilogram are without appreciable effect upon the course of *Tr. equiperdum* infections of rats.

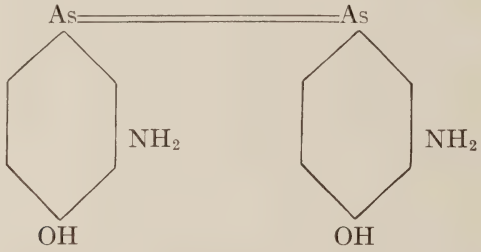
Phenylglycin-p-arsonic Acid and P-arsenophenylglycin.—As will be discussed shortly Ehrlich explained the parasiticidal activity of atoxyl on the basis that in the body it was reduced to the more active trivalent form and this led him to study other aromatic compounds in which the arsenic is trivalent, resulting in the discovery of phenylglycin-p-arsonic acid, an important derivative of arsanilic acid. On reduction of this substance by sodium hydrosulphite, a trivalent compound called *p-arsenophylglycin* is obtained representing the arsenobenzol type of organic arsenical compounds. In this type two arsenic atoms are linked together by a double bond, and each is coupled to the benzol nucleus by a single linkage.

In phenylglycin-p-arsonic acid the arsenic is in pentavalent form; Voegtlin and Smith found the lethal dose for rats by intravenous injection to be about 2.06 gm. per kilogram of body weight and the curative dose for *Tr. equiperdum* infections about 1.03 gm. per kilogram giving a chemotherapeutic index of about 2. The trivalent compound p-arsenophenylglycin is more active than its sodium salt, sodium-p-arseno-phenyl-glycinate, number 418 in Ehrlich's series, and appearing under the name of "spirasyl." Roehl,[14] Tsuzuchi and Voegtlin and Smith have studied its toxicity and trypanocidal effects and the latter found that it possessed a chemotherapeutic index of 16, which is quite high.

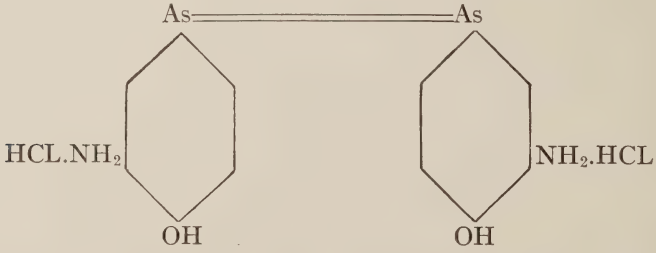
Neurotropism of Arsenical Compounds in Mice.—Mention may be made at this time of the peculiar series of symptoms sometimes produced in mice

by the administration of some of the pentavalent arsenical compounds first described in 1907 by Mesnil, Nicolle and Aubert[15] in rats treated with atoxyl. Browning states that this condition was particularly likely to develop after the administration of arsacetin to mice. General muscular tremors develop and especially characteristic is a jerking hyper-extension of the head, causing the animals to look up into the air. Three or four days later the mice begin to behave in a manner indistinguishable from that of Japanese dancing-mice. In their glasses or on the open floor they go round in circles for hours at a stretch and seem to prefer one direction making only occasionally a few reverse turns. This condition may persist indiminished in animals under observation for many months and is apparently due to the effects of arsenic upon the higher nerve centers as discussed in Chapter LI.

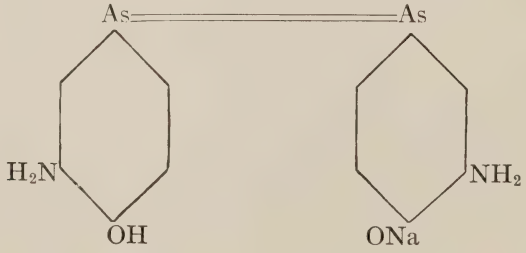
Toxicity and Trypanocidal Activity of Arsphenamin (Salvarsan).—By further varying and changing the position and kind of sidechains, Ehrlich and Bertheim finally produced a new compound, the 606th of their series. The base of this substance, is 3, 3-diamino- 4, 4-dihydroxyarsenobenzene and the dihydrochlorid is the salt employed in the treatment of syphilis, being commonly designated as "salvarsan," "606" or "arsphenamin," the chemical constitution of the base being as follows:



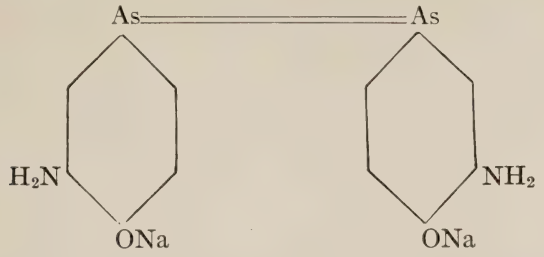
The dihydrochlorid is the form of arsphenamin or salvarsan that appears on the market, its chemical constitution being as follows:



When dissolved in water the solution of this substance is acid and highly toxic; before administration, therefore, the solution requires neutralization with sodium hydroxid. If just enough alkali is added to effect neutralization the mono-sodium salt is produced:



A slight excess of alkali produces the disodium salt, and in this form arsphenamin (salvarsan) is least toxic:



But few medicinal compounds have been studied as extensively as arsphenamin both from the standpoint of toxicity for man and the lower animals

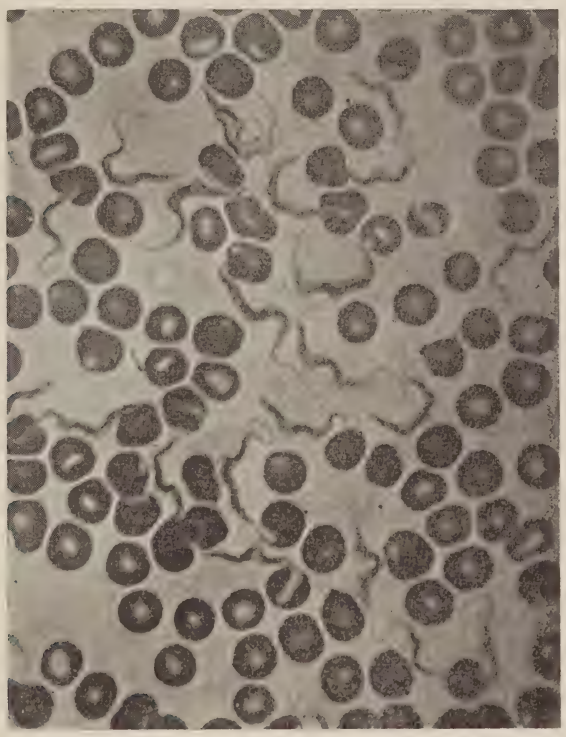


FIG. 12.—BLOOD OF RAT WITH TRYPANOSOMIASIS.
Film prepared four days after intraperitoneal inoculation with *Tr. equiperdum.*

and for curative activity in experimental trypanosomiasis especially in the mouse and rat.

Toxicity for human beings is discussed in more detail in Chapters XLV and XLVI, but here it may be stated that when alkalinized (disodium) solutions of arsphenamin are administered to white rats by intravenous injection as described in Chapter II, the drug is well tolerated in amounts of 0.120 gm. per kilogram of weight. It is required by the U. S. Public Health Service that every lot of arsphenamin employed in the treatment of syphilis shall be borne by white rats for two days, in at least this amount; as a matter of

fact, however, in most instances the arsphenamin being prepared in this country at present is tolerated by rats in doses ranging from 0.120 to 0.150 gm. per kilogram and in exceptional instances even in slightly larger amounts.

Trypanocidal tests for measuring the therapeutic activity of arsphenamin (salvarsan) were first employed (1915) on a large scale in this country by the writer in coöperation with Schamberg and Raiziss.

In general terms the trypanocidal activity of alkalinized (disodium) solutions of arsphenamin (salvarsan) as determined by my method for *Tr. equiperdum* infections of rats is approximately 0.002 to 0.003 gm. per kilogram of weight (Figs. 12 and 13). In a study by Schamberg, Raiziss and

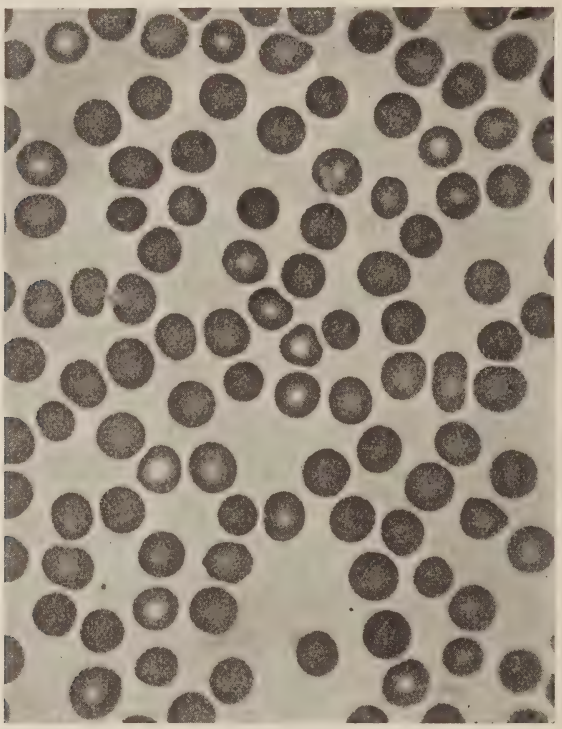


FIG. 13.—TRYPANOCIDAL ACTIVITY OF ARSPHENAMIN.
Blood of same rat shown in Fig. 12 twenty-four hours after the intravenous injection of arsphenamin.

myself[16] of the trypanocidal activity of the arsphenamin (salvarsan) prepared by six different laboratories including the original Ehrlich-Hata preparation as well as that prepared by ourselves, the average trypanocidal doses per kilo of weight varied from 0.0018 to 0.0026 gm. per kilogram.

At the time these tests were made (1919) the average toxicity of the products of different laboratories for white rats was approximately 0.105 gm. per kilogram of body weight giving the following chemotherapeutic ratio:

$$\frac{\text{Maximum tolerated dose: 0.105 gm. per kilogram}}{\text{Minimal curative dose: 0.0023 gm. per kilogram}} = 45.6$$

At the present time (1925) the toxicity of arsphenamin tends to be somewhat less than in former years, averaging about 0.130 gm. per kilogram of body weight and yielding a chemotherapeutic index of about 50.

In the Hygienic Laboratory method of Voegtlin and Smith the tests are likewise conducted with white rats and *Tr. equiperdum* but the infection is allowed to become heavier before treatment is given than in my method; consequently the amounts of arsphenamin required to effect sterilization are greater. Furthermore, in my method the trypanocidal dose is recorded as the smallest amount of compound effecting complete sterilization of the animal whereas in the Hygienic Laboratory method the minimal effective dose is the smallest amount ridding the blood of trypanosomes within twenty-four to forty-eight hours after administration as determined by microscopical examinations. Sometimes however, trypanosomes reappear and destroy the rat so that the minimal effective dose in the Hygienic Laboratory method is not necessarily the minimal curative dose in the meaning of my method. The two methods, however, are somewhat comparable in results, the minimal effective dose by the Hygienic Laboratory method being in general terms about 2.4 to 4 times larger than the minimal curative dose in my method.

In a study of the relative trypanocidal value of arsphenamin (salvarsan) of different manufacture by Voegtlin and Smith[17] the average minimal lethal dose was found to be 53.2 c.c. and the average minimal effective dose 3.1 c.c. per kilogram, both values being expressed in terms of cubic centimeters of 1:100 arsenic equivalent solution. These values are approximately equivalent to 0.116 gm. of arsphenamin per kilogram as the minimal lethal dose and 0.007 gm. per kilogram as the minimal effective dose giving a chemotherapeutic ratio of 17.

Trypanocidal Activity of Arsphenamin and Neoarsphenamin for the Guinea-Pig and Rabbit.—As described in Chapter X, trypanosomiasis in the guinea-pig and rabbit pursues a much different course than in the mouse and rat in that trypanosomes are found in the blood only during certain cyclic periods, the infection being essentially an involvement of the tissue and running a more chronic course. These features are especially characteristic of trypanosomiasis of the rabbit.

In order to determine the curative dose of arsphenamin and neoarsphenamin in these infections, it is necessary to keep the animals under observation for several months in order to make sure that relapses do not occur. For this reason trypanocidal tests employing guinea-pigs and rabbits are not as serviceable as tests employing rats for the routine testing of these compounds and very little use has been made of them; on the other hand, trypanosomiasis in these animals more closely approaches the course of syphilis in the human being by reason of the predominance of chronic tissue changes and the tests are of distinct service for gaining information on the trypanocidal activity of medicaments under these conditions.

The toxicity of arsphenamin and neoarsphenamin for guinea-pigs and rabbits by intravenous injection is not much different from that for rats when administered in exactly the same manner. The minimal trypanocidal doses for twenty-four hour infections is however, slightly higher for guinea-pigs and decidedly higher for rabbits, so that the chemotherapeutic indices are somewhat lower than observed in rat tests.

When the infection is allowed to continue in guinea-pigs for a week before treatment is given the minimal curative doses of both arsphenamin and neoarsphenamin are about 5 times more than required for twenty-four hour infections; these larger amounts are due to the greater difficulty of effecting sterilization of the tissues.

In the rabbit, the amount required to cure twenty-four hour infections is higher per body weight than required for similar infections of guinea-pigs and rats, but in the treatment of older infections (1 to 6 weeks) 2 to 3 times larger

doses are required to effect complete sterilization of the blood and fixed tissues.

Variation in Trypanocidal Activity and Keeping Qualities of Arsphenamin (Salvarsan).—From the chemical standpoint arsphenamin is quite a stable compound and when the powder is properly preserved, toxicity and trypanocidal activity remain constant to a remarkable degree over long periods of time. I have included one lot of arsphenamin in all trypanocidal tests of new compounds for almost a period of three years for purposes of comparison to express the trypanocidal action of new compounds in terms of the "arsphenamin index," ratio or coefficient, and the results have been quite uniform.

Different lots of arsphenamin prepared by the same manufacturer may vary to a slight degree in trypanocidal activity but marked variations are generally due to changes in the trypanosomes. In the manufacture of arsphenamin on a commercial scale the total output of a day is generally mixed into one large lot which tends to minimize slight variations in the toxicity and trypanocidal activity of individual lots. On several occasions I have laid aside a few ampules from time to time and after collecting these over periods of four or five months have tested all at the same time for trypanocidal activity; in this manner variations due to the trypanosomes were controlled and differences in trypanocidal activity ascribed to the compounds. In experiments of this kind the trypanocidal activity of arsphenamin has been found quite constant, an experiment conducted in 1919 with twelve different lots yielding minimal curative doses varying from 0.006 to 0.008 gm. per kilogram of body weight. In 1920–1921 a similar experiment yielded values varying from 0.003 to 0.005 gm. per kilogram and a third experiment 0.002 to 0.004 gm. per kilogram.

However, marked differences in trypanocidal activity are easily caused by variations in the resistance of the strain of trypanosomes. On at least four occasions during the past six years strains of *Tr. equiperdum* employed in my laboratory have been rendered arsenic resistant and whereas rats infected with normal strains were sterilized with doses of arsphenamin ranging from 0.002 to 0.004 gm. per kilogram, rats infected with the resistant strains required doses as high as 0.015 to 0.020 gm. per kilogram. On the other hand rats infected with normal strains taken from guinea-pigs or rabbits are likely to be sterilized with slightly smaller amounts than required for normal strains propagated in rats. I have, however, discussed these points in more detail in relation to the "arsphenamin index," but mention them here again to emphasize that apparent variations in the trypanocidal activity of arsphenamin may be more readily caused by variations in the resistance of the trypanosomes to the effects of arsphenamin than to the compound itself.

The arsphenamin (salvarsan) prepared in different laboratories yields approximately the same general average trypanocidal values.

In 1921, I again tested 20 different lots from six different laboratories including products prepared in Germany, Canada and the United States; the results showed the average minimal curative doses to range from 0.0018 to 0.0026 gm. per kilogram of rat. Kolle[18] has observed that in mice the maximum tolerated doses of salvarsan, silver, gold and platinum salvarsans have been from 0.1 to 02.5 gm. per kilo with minimal curative doses for *Tr. brucei* infections from 0.008 to 0.025 gm. per kilogram.

Toxicity and Trypanocidal Activity of Acid, Neutral and Alkalinized Solutions of Arsphenamin (Salvarsan).—Acid solutions of arsphenamin, that is, solutions of the dihydrochlorid in water without neutralization with sodium hydroxid, are from 4 to 6 times more toxic than solutions of the same compound after neutralization. Trypanocidal tests have shown that the

curative activity of acid solutions is likewise greater than neutralized and alkaline solutions, but the chemotherapeutic indices are lower; this is due to the striking influence of alkalinization upon toxicity for the animal host. The maximum tolerated dose of arsphenamin in acid solution is about 0.010 to 0.030 gm. per kilogram while the same compound in neutral (monosodium) or alkaline (disodium) solution is tolerated in doses of 0.120 to 0.150 gm. per kilogram. In other words, acid solutions are more toxic for both trypanosomes and test animal than neutral or solutions, but while the toxicity of arsphenamin in acid solution for the parasites is double that of the same compound in alkaline solution, the toxicity for the animal is 7 to 15 times higher.

Furthermore, it would appear that the chemotherapeutic indices of neutral (monosodium) solutions of arsphenamin are somewhat higher than alkaline (disodium) solutions. Adding about one third excess of sodium hydroxid to a neutral solution of arsphenamin and thereby converting it into an alkaline or disodium solution, may reduce toxicity for the trypanosomes (trypanocidal activity) to a greater degree than reduction of toxicity for the animal and thereby reduce the chemotherapeutic index of alkaline solutions. In one experiment conducted in 1919 the indices were as follows:

Acid solution	Maximum tolerated dose: 0.010–0.020 gm. Minimal curative dose: 0.003 gm.	= index of 3 to 6.
Neutral Solution	Maximum tolerated dose: 0.120–0.140 gm. Minimal curative dose: 0.003 gm.	= index 40 to 46.
Alkaline Solution	Maximum tolerated dose: 0.140–0.150 gm. Minimal curative dose: 0.004 gm.	= index 35 to 38.

In practice, however, alkaline solutions are to be preferred for intravenous injections in the treatment of syphilis, because they are somewhat safer and less likely to produce reactions.

Trypanocidal Activity of Shaken Solutions of Arsphenamin (Salvarsan).— It is a well-established fact that oxidation of arsphenamin increases the toxicity by conversion of a part of the drug into the more toxic amino-hydroxy-phenyl-arsenoxid (arsenoxid). This observation was early insisted upon by Ehrlich and his associates, who urged against unnecessary shaking of solutions of arsphenamin and upon the prompt administration of the drug after solutions were prepared to prevent the formation of this substance.

Schamberg, Raiziss and myself[19] have studied the effects upon trypanocidal activity of shaking acid and alkalinized solutions of arsphenamin marketed by three different laboratories. When 10 c.c. of 2 per cent. solutions in warm water were placed in 25 c.c. cylinders and shaken for one minute (about 250 times) followed by alkalinization in the usual manner and tested for trypanocidal activity, it was found that the solutions of some products sometimes sterilized rats in slightly smaller amounts than required of solutions that were not shaken beyond the time required for solution. However, when solutions were first alkalinized and then shaken for one and ten minute periods, trypanocidal activity was considerably increased.

Chemical examinations to determine quantitatively the oxygen requirements of the arsphenamins under these conditions on the basis that the trivalent arsenic takes up iodin quantitatively followed by oxidization to the corresponding arsonic acid derivative, have shown that when alkalinized solutions were shaken the oxygen requirement was decreased about 17.4 per cent. This indicated the production of arsenoxid which probably accounts for the solutions being rendered more trypanocidal by shaking. Voegtlin and Smith[20] believe that the therapeutic activity of arsphenamin is due to the formation of oxidation products in the body after injection, and the results of these experiments lends confirmation to these views. I will,

however, discuss this phase shortly in a consideration of the mechanism of trypanocidal activity of arsphenamin and neoarsphenamin.

Arsenoxid production, however, was not produced by shaking acid solutions of arsphenamin to as great degree as in alkalinized solutions and for this reason the shaking ordinarily required for the preparation of solutions of arsphenamin for administration in the treatment of syphilis does not materially influence either the toxicity or trypanocidal activity and particularly since the required shaking is done before alkalinization. Similar changes occurred with solutions of neoarsphenamin.

Trypanocidal Activity of Preserved Solutions of Arsphenamin; Influence of Standing upon Trypanocidal Activity.—Solutions of arsphenamin exposed to the air usually become darker in color and acquire an increased toxicity. These changes are likewise alleged to be due to oxidation and the prompt administration of the solutions has been insisted upon and emphasized. As a general rule the rapidity and degree of these changes is in proportion to the square surface of the solution exposed to the air, that is, they occur more rapidly in an Erlenmeyer flask than in a narrow cylinder. Solutions kept in an oxygen-free atmosphere undergo changes of this kind very slowly and toxicity may remain unaltered for relatively long periods of time. Changes in trypanocidal activity of alkalinized solutions of arsphenamin exposed to air are very peculiar. At first a slight increase of trypanocidal activity may occur followed by a marked decrease.

When alkalinized solutions are kept in cylinders filled to the stoppers, the color changes occurred much more slowly as likewise changes in trypanocidal activity; apparently retardation of these changes was due to the much smaller surface of the solutions exposed to air.

Alkalinized solutions of arsphenamin kept in high vacuum do not usually show any color changes or increase of toxicity for periods of several months and indeed, for a year or more in some instances. *A gradual reduction in trypanocidal activity,* however, may take place and for this reason preserved solutions of arsphenamin should not be kept indefinitely when employed for the treatment of syphilis. On the basis of this gradual loss of trypanocidal activity, which is parallel with loss of spirocheticidal properties, the Hygienic laboratory requires that such solutions shall not be older than six months when employed in the treatment of syphilis.

We have found similar changes in trypanocidal activity with solutions of neoarsphenamin, described later in this chapter. Whether or not the physical changes in these solutions embracing a darkening in color with the formation of precipitates and increase of toxicity are due to the formation of arsenoxid, cannot be definitely stated although under the circumstances one naturally surmises that oxidation products are responsible. As discussed in Chapter XLVI, the toxicity of these solutions may not be increased or an increase of toxicity may be followed by a decrease suggesting in some instance the gradual production of the pentavalent amino-hydroxy-phenyl arsonic acid. The same is sometimes true of solutions kept in contact with air, that is, a slight increase of trypanocidal activity may be detected followed by a progressive decrease.

Trypanocidal Activity of Solutions of Arsphenamin (Salvarsan) Prepared with Water at Varying Temperatures.—Of further interest in this connection is the influence upon trypanocidal activity of the temperature of the water employed for preparing solutions of arsphenamin. Ordinarily water at room temperature (approximately 22°C.) is employed, but higher temperatures may be required in order to effect complete solution of the powder with a minimum degree of shaking.

I have conducted trypanocidal tests with the arsphenamins prepared by three different laboratories by preparing three solutions of each with water at 20°–22°C., 70°C. and 100°C. After alkalinization in the usual manner these were injected into infected rats according to the technic of my trypanocidal tests; the results have shown that the arsphenamin prepared by two laboratories tended to loose slightly in trypanocidal activity when boiling water (100°C.) was employed in preparing the solutions; ordinary hot water (70°C.) had no appreciable effect. With the arsphenamin prepared in a third laboratory, which was slowly soluble in cold water, the use of hot and boiling water had no effect upon trypanocidal activity. Why these varying results occurred with the arsphenamins of different manufacture I am unable to state; as discussed in Chapter XLVI, solutions prepared with water at 70°C. and 100°C. were almost always less toxic than solutions prepared with water at 22°C. indicating that heat tends to reduce the toxicity of arsphenamin for both host (the test animal) and parasites (*Tr. equiperdum*).

Toxicity and Trypanocidal Activity of Sodium Arsphenamin (Sodium Salvarsan).—Since the disodium salt of arsphenamin, prepared by alkalinizing solutions with sodium hydroxid, does not keep well and requires almost immediate administration, Ehrlich prepared a stable sodium salt known as "1206" in his series and possessing a chemical structure essentially the same as arsphenamin.

The substance when dissolved in water yields a neutral or slightly alkaline solution ready for administration. It has been extensively employed in Europe and to a lesser extent in this country, for the treatment of syphilis although extensive studies of its curative activity for experimental infections do not appear to have been made.

Schamberg, Raiziss and myself[21] have studied the toxicity and trypanocidal activity of the sodium arsphenamin prepared by two different laboratories. The toxicity of four different lots from one of these laboratories varied from 0.190 to 0.250 gm. per kilo with an average of 0.212 gm.; the minimal curative doses for *Tr. equiperdum* infections varied from 0.020 to 0.030 gm. per kilo with an average of 0.024 gm. and yielding a chemotherapeutic index of 8.8.

The toxicity of eight different lots from a second laboratory likewise varied from 0.190 to 0.250 gm. per kilo with an average of 0.215 gm.; the minimal curative doses varied from 0.010 to 0.020 gm. with an average of 0.016 gm. and yielding a chemotherapeutic index of 13.4.

At the time these tests were made the average minimal curative dose of arsphenamin was 0.005 gm. per kilo and neoarsphenamin about 0.009 gm. Sodium arsphenamin, therefore, was from 3 to 5 times less trypanocidal than arsphenamin and 2 to 3 times less trypanocidal than neoarsphenamin. Since neoarsphenamin is less toxic, more trypanocidal and just as easy of administration as sodium arsphenamin, the former has been more extensively employed in the treatment of syphilis.

Toxicity and Trypanocidal Activity of Phospharsphenamin (Galyl).—This substance, which is manufactured in France and employed in the treatment of syphilis especially by French clinicians, is said to be an arsphenamin derivative containing phosphorous, 4.4′ dihydroxyarsenobenzene 3.3′ phosphamic acid or No. 1116 of Mouneyrat's series. In the United States it is licensed under the official name of "phospharsphenamin," its trade name being "galyl."

Phospharsphenamin is supposed to contain 35.3 per cent. arsenic and 7.5 per cent. phosphorus. It occurs as an acidic substance, insoluble in water, and dissolves in aqueous sodium carbonate. It is dispensed sealed up in

ampules containing the dry sodium carbonate, so that it readily dissolves upon the addition of water. Solutions made in this way are yellow, or greenish yellow, the greenish color rapidly deepening upon standing.

A new "galyl" has been recently distributed which does not require alkalization. A study of this substance by the chemical laboratory of the American Medical Association in 1922 showed that it responds to practically all the tests for sodium arsphenamin except that there is present both sulphite and phosphate. The Council on Pharmacy and Chemistry concluded that when the new galyl is dissolved in water, either it is partly decomposed into phosphate and sodium arsphenamin or else the original product contains sodium arsphenamin and sodium phosphate. In either case the injection will probably amount to the administration of a mixture of phospharsphenamin (if any is present), sodium arsphenamin, sodium phosphate, sodium sulphite and sugar.

In my laboratory the average maximum tolerated dose of phospharsphenamin (galyl) for white rats by intravenous injection was about 0.350 gm. per kilogram of body weight; the minimal curative dose for twenty-four-hour *Tr. equiperdum* infections was 0.085 gm. per kilo giving a chemotherapeutic index of 4.1. According to Kolle[22] the maximum tolerated dose of galyl for mice is 0.500 gm. per kilo and the minimal curative dose for *Tr. brucei* infections about 0.165 gm. per kilo giving a chemotherapeutic index of 3.

Toxicity and Trypanocidal Activity of Silver Sodium Arsphenamin (Silver Salvarsan).—In 1912 Ehrlich and his associates succeeded in synthesizing a new series of compounds in which different molecular proportions of the salts of heavy metals enter into intimate combination wtih the arsenobenzol compounds. A short time afterward, Danysz,[23] working independently, announced the discovery of metallic complexes from arsphenamin and silver nitrate and since then, silver sodium arsphenamin or silver salvarsan, as it is commonly designated, has been widely employed in the treatment of syphilis.

In 1915 Ehrlich and Karrer[24] published more details concerning aromatic compounds of trivalent arsenic combined with complex salts of heavy metals which were characterized by deep colors and great stability. These observations included all arseno compounds, as well as arsenoxids and arsines under certain conditions, and all salts of copper, silver, gold, mercury, palladium, platinum, iridium, ruthenium, and osmium. According to Ehrlich and Karrer the metal may be bound by the orthoamino phenol group, by both the phenol and arseno groups, or the residual affinity of the arsenic group may contain the metal; however, the silver product described by them in 1915 is not of the same composition as the silver sodium arsphenamin now employed in the treatment of syphilis. The exact chemical composition of this substance is unknown but it is regarded by Myers[25] as the sodium salt of the monosilver 3-3′ diamino—4—4′ dioxy-arseno-benzene.

According to Kolle,[26] who has extensively studied the biological properties, silver arsphenamin should contain 22.4 per cent. arsenic and 14.1 per cent. silver; analyses show, however, that the arsenic content may vary from 19 to 21.5 per cent. and the silver from 13.8 to 15.7 per cent.

The toxicity of the compound ranges from 0.090 to 0.120 gm. per kilogram of rat by intravenous injection in the usual standard method, and in the United States, the Hygienic Laboratory requires that products for use in the treatment of syphilis shall be borne by rats in a minimum dose of 0.120 gm. per kilogram.

I have determined the minimal curative dose of different lots of silver sodium arsphenamin prepared in two different laboratories during 1920 and

1921; the average minimal curative dose was approximately 0.0028 gm. per kilo as compared with 0.004 of arsphenamin. In 1922, however, the trypanocidal activity of silver sodium salvarsan was found to be about 0.002 gm. per kilo and arsphenamin approximately 0.003 gm. Inasmuch as the toxicity of this arsphenamin was about 0.120 gm. per kilo of rat and the acceptable lots of silver sodium arsphenamin about 0.110 gm., the chemotherapeutic index of the silver compounds was 55, as compared with 40 for the index of arsphenamin.

According to Kolle[27] the maximum tolerated dose of silver arsphenamin (silver salvarsan) for mice is about 0.250 gm. per kilo and the minimal curative dose for *Tr. brucei* infections about 0.011 gm. per kilo giving a chemotherapeutic index of 22.

Toxicity and Trypanocidal Activity of Sulfarsenol (Sulpharsphenamin).— Within the past few years another organic arsenical has been introduced for the treatment of syphilis designated as *sulfarsenol*. Ehrlich and his associates in 1912 described a compound with the substitution of an amino radical of arsphenamin by a methylene sulphonic acid group and the compound "sulfarsenol" prepared by Lehnhoff-Wyld appears to be identical in structure. In 1922, Voegtlin, Johnson and Dyer[28] prepared the same compound, but differing from the German method in that they begin with arsphenamin in a water-alcohol solution, work at ordinary temperature and precipitate the final disodium salt with alcohol in one continuous operation, for which they proposed the name *sulpharsphenamin*. Both names therefore, apparently designate the same compound which occurs as a light, yellowish power freely soluble in cold water. Unlike arsphenamin and neoarsphenamin, when injected intramuscularly, it has been found to produce but slight local irritant properties and has been established as a remedy of choice in the treatment of syphilis when subcutaneous or intramuscular injections are indicated.

The arsenic occurs in from 20.7 to 23.6 per cent. and sulphur in from 8.7 to 12.8 per cent., the atomic ratio of arsenic to sulphur ranging from 1:0.91 to 1:1.45. The compound differs from neoarsphenamin by the presence of one additional atom of oxygen.

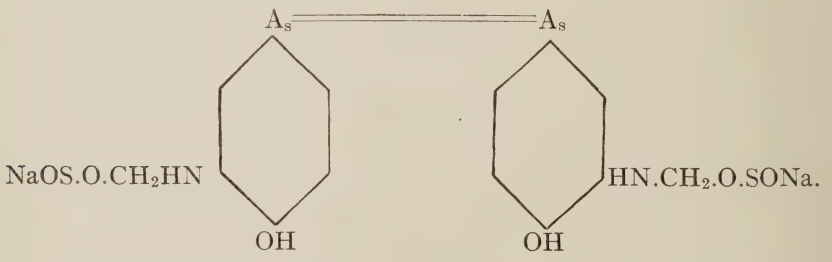
Levy-Bing, Lehnhoff-Wyld and Gerbay[29] gave 0.400 gm. per kilo as the maximum tolerated dose for mice; they do not appear to have made trypanocidal tests. According to Voegtlin, Johnson and Dyer the toxicity of sulpharsphenamin for white rats by intravenous injection was approximately 0.395 gm. per kilo and by subcutaneous injection approximately 0.530 gm. The minimal effective dose as determined by the Hygienic Laboratory method with rats infected with *Tr. equiperdum* was approximately 0.021 gm. per kilo when administered intravenously and 0.024 gm. by subcutaneous injection. As shown by these investigators, the full effects of sulpharsphenamin may require 3 or 4 days whereas both arsphenamin and neoarsphenamin act in 1 to 2 days. This slower action on the part of sulpharsphenamin, which is probably due to a slower rate of oxidation in the body to the trivalent arsenious oxid compounds, may constitute however, a desirable feature of the drug in the treatment of some cases of syphilis when slow but prolonged therapeutic activity is indicated. According to Myers and Corbitt[30] sulpharsphenamin by intravenous injection to rats is at least two and a half times less trypanocidal than good brands of neoarsphenamin. Raiziss, Severac and Moetsch[31] employing the Voegtlin trypanocidal method, found the minimal effective dose to be about 18 mgm. per kilogram as compared with 4 mgm. of arsphenamin or 6 mgm. of neoarsphenamin; practically the same results were observed by both intramuscular and intravenous routes of administration.

In my laboratory the average maximum tolerated dose of sulpharsphenamin kindly furnished by Dr. Voegtlin was 0.310 gm. per kilo when administered to rats intravenously and 0.400 gm. per kilo by subcutaneous injection. The minimal curative dose in *Tr. equiperdum* infections by intravenous injection according to my method was 0.020 gm. per kilo of rat and 0.015 gm. per kilo by subcutaneous injection. Sulpharsphenamin prepared by a second laboratory showed a maximum tolerated dose of 0.450 gm. per kilo intravenously and 0.600 gm. by subcutaneous injection; the minimal curative dose by subcutaneous injection was 0.010 gm. per kilo and 0.030 gm. intravenously showing that sulpharsphenamin is least toxic and more trypanocidal by subcutaneous than by intravenous injection.

It was observed that when sulpharsphenamin was administered intravenously that its chemotherapeutic index was equal to or but slightly less than the index for arsphenamin by intravenous injection; the index for sulpharsphenamin was however, much less than that of neoarsphenamin by this route of administration.

However, when sulpharsphenamin is given subcutaneously the chemotherapeutic index is increased, because its toxicity is lower while its trypanocidal activity is increased by this route of administration. These effects are probably due to the greater facility for oxidation in the tissues with the production of therapeutically active "oxids" and indicates quite clearly that the maximum degree of curative activity of sulpharsphenamin is to be gained by administering the compound by subcutaneous or intramuscular injection.

Toxicity and Trypanocidal Activity of Neoarsphenamin (Neosalvarsan).—Impressed with the dangers attending the accidental administration of acid solutions of arsphenamin and desirous of simplifying the administration by evolving a substance that does not require neutralization before injection, Ehrlich continued his investigations with the arsenobenzol compounds and finally discovered a new compound sodium 3,3′—diamino-4,4′-dihydroxy-arseno-benzene—N—methylene sulphinate, designated as "neosalvarsan" or "914." This substance is now known officially in the United States as "neoarsphenamin" and while the exact formula is unknown, the following structure is generally assigned:



Since neoarsphenamin contains on the average 19 per cent. arsenic as compared with arsphenamin which contains approximately 30 per cent., the curative activity of the former is less; according to Ehrlich and Hata the ratio of therapeutic activity is such that 0.6 gm. of arsphenamin is equivalent to 0.9 gm. of neoarsphenamin as based upon therapeutic experiments on hen spirillosis and mice infected with the spirochetes of relapsing fever.

In 1919, Schamberg, Raiziss and myself[32] determined the trypanocidal activity of neoarsphenamin prepared by six different laboratories including the German product. The general average trypanocidal dose for all com-

pounds was 0.004 gm. per kilogram of rat, neoarsphenamin being therefore, about 1.7 times less trypanocidal than arsphenamin. Under these conditions the equivalent of 0.6 gm. of arsphenamin is 1.05 gm. neoarsphenamin in the treatment of experimental trypanosomiasis.

During 1920–1921, however, the trypanocidal activity of one of the American-made products was increased and not infrequently proved almost as trypanocidal as arsphenamin.

Similar results were observed by Voegtlin and Smith[33] in a comparative study of the neoarsphenamins prepared in different laboratories, insofar as concerns the ratio of trypanocidal activity between arsphenamin and neoarsphenamin. The general average of trypanocidal activity was 3.4 c.c. of 1:100 arsenic equivalent solution per kilogram of rat; with the arsenic content at 18.9 per cent. this amount was equivalent to about 0.0079 gm. neoarsphenamin per kilogram, which is larger than required in my test because of the larger number of organisms requiring destruction. The ratio of trypanocidal activity, however, between arsphenamin and neoarsphenamin was 0.6:1.02 gm. which was quite similar to that found by Schamberg, Raiziss and myself.

In a study of the trypanocidal activity of German and English-made neoarsphenamins by Dale and White, assisted by Burn, Dushan and Marchal,[34] the minimal curative doses in 72-hour periods of observation varied from 0.015 to 0.020 gm. per kilogram of mouse with a general average of 0.020 gm. The general average for arsphenamin was 0.010 gm. per kilogram so that the ratio of trypanocidal activity was 0.6:1.2 gm. being slightly higher than observed by Voegtlin and Smith and myself.

Variations in Trypanocidal Activity of Neoarsphenamin (Neosalvarsan).— From the chemical standpoint neoarsphenamin is a more unstable compound than arsphenamin. Different lots prepared by the same manufacturer are more apt to vary in the biological properties of toxicity and trypanocidal activity than arsphenamin; the drug is likewise more likely to deteriorate in the ampules than arsphenamin.

On several occasions I have tested lots of twelve different samples of neoarsphenamin at one time in order to equalize the variations due to the organism and have found that the trypanocidal activity may vary all the way from minimal curative doses of 0.001 gm. to 0.010 gm. per kilogram of body weight although the great majority of products fell within the limits of 0.002 to 0.004 gm. per kilogram of rat.

Voegtlin and Smith in the Hygienic Laboratory have also found wide variations in the minimal effective doses of the neoarsphenamin of different manufacture and have called particular attention to this fact in relation to the use of neoarsphenamin in the treatment of syphilis, to which I will refer later in more detail. These investigators found that the trypanocidal activity of neoarsphenamin of different manufacture may vary from 2.5 to 4.0 c.c. of 1:100 arsenic equivalent solution per kilogram of rat equivalent approximately to 0.005 to 0.010 gm. neoarsphenamin per kilo, with an average of 3.4 c.c. of 1:100 arsenic equivalent solution or about 0.007 to 0.008 gm. neoarsphenamin per kilo.

Dale and White report that the British-made neoarsphenamin had at first a minimal curative dose of 0.040 to 0.050 gm. per kilogram of mouse which later the manufacturers improved upon to the extent that the dose became 0.010 to 0.020 gm. per kilogram.

According to Kolle,[35] the maximum tolerated dose of neoarsphenamin (neosalvarsan) is about 0.375 gm. per kilo and the minimal curative dose for mice infected with *Tr. brucei* about 0.040 gm. per kilo giving a chemotherapeutic index of 9.

Keeping Qualities of Neoarsphenamin.—As previously stated the powder sealed in ampules from which the air has been excluded either by the production of a vacuum or by displacement of the air with a monoxidizing gas such as carbon dioxid, nitrogen, or hydrogen, tends to hinder physical and biological changes. Roth[36] has reported that a study of neoarsphenamin prepared by four different laboratories showed that changes may occur in color, solubility, toxicity, mobility in ampule, and odor after varying periods up to three years.

I have also studied the question of changes in trypanocidal activity and toxicity by testing the same product of three different laboratories at intervals of a month over a period of one year, the first tests being conducted in from three to four weeks after the manufacture of the respective products. Each lot was kept at room temperature and tested against the same strain of *Tr. equiperdum.* At the same time observations were made on the toxicity and such physical changes as alterations in color of the solutions, solubility, odor, etc. During this period no changes in solubility, color or odor occurred with any of the compounds; it was also noted that changes in trypanocidal activity did not occur in nearly the same degree as changes in toxicity. A slight tendency to deterioration in trypanocidal activity was apparent over this period of one year but the changes were neglible and within the range of experimental error.

Trypanocidal Activity of Shaken and Preserved Solutions of Neoarsphenamin (Neosalvarsan).—Neoarsphenamin is ordinarily so readily soluble in water that the influence of shaking the solution does not enter as a possible factor influencing its toxicity and trypanocidal activity. However, some products are not as readily soluble as others and may require considerable shaking which, under certain circumstances, may result in physical and biological changes.

The influence upon toxicity of shaking solutions of neoarsphenamin in water, is discussed in Chapter XLVI. Schamberg, Raiziss and myself[37] have also studied the effects upon trypanocidal activity with practically negative results. Two per cent. solutions in water shaken vigorously for one minute (10 c.c. in a 25 c.c. cylinder for approximately 250 times) did not reduce or increase trypanocidal activity; when shaken for ten minutes the trypanocidal activity of the products of two laboratories was very slightly decreased, as shown by the late development of trypanosomiasis.

When solutions of neoarsphenamin in water are kept at room temperature a loss in trypanocidal activity occurs, as is true of solutions of arsphenamin. The degree and rapidity of this loss in curative activity is proportional to the square surface of exposure to air and more rapid than in the case of arsphenamin. Inasmuch as there is no practical end to be served by the preservation of solutions of neoarsphenamin over long periods of time in view of the ease of preparing fresh solutions as required, I have never conducted similar studies with solutions preserved in high vacuum as have been described with solutions of arsphenamin; I surmise, however, that similar changes would be observed in the nature of a gradual decrease in trypanocidal activity after the elapse of several months.

Trypanocidal Activity of Solutions of Neoarsphenamin (Neosalvarsan) Prepared with Water at Varying Temperatures.—Of more practical importance is the influence upon trypanocidal activity that may be exerted by the temperature of the water employed for preparing solutions of neoarsphenamin for administration in the treatment of syphilis. Ordinarily water at room temperature (about 22°C.) is employed owing to the readily soluble nature of the compound.

The results of comparative trypanocidal tests with the neoarsphenamin prepared by three different laboratories, each tested at the same time dissolved in water at 22°C., 70°C. and 100°C. have shown that boiling water tends to slightly reduce the trypanocidal activity of most samples, while ordinary hot water (70°C.) has no appreciable effects.

Rapidity of Trypanocidal Activity of Arsphenamin and Neoarsphenamin. As shown by Voegtlin and Smith, the intravenous injection of minimal curative doses of arsphenamin and neoarsphenamin is followed almost at once by a decrease of the number of parasites in the blood until in from four to six hours and almost certainly within twenty-four hours after injection, none are to be found in the peripheral blood. According to these investigators this immediate trypanocidal action is especially characteristic of arsenicals belonging to the arsenoxid group, that is, trivalent compounds capable of being rapdily oxidized in the body.

I have likewise observed essentially similar results. Both arsphenamin and neoarsphenamin produced an agglutination of the organisms in the blood, which interfered with the accuracy of the counts. With the larger doses this phenomenon did not occur but rapid reduction in the numbers of parasites was produced resulting in sterilization in about six hours after injection. I have not seen any reference in the literature to the agglutination of trypanosomes by sub-curative doses of arsphenamin and neoarsphenamin and it is highly probable that the phenomemon is part of the mechanism concerned in the trypanocidal activity of these compounds.

Trypanocidal Activity of Mutiple Doses of Arsphenamin and Neoarsphenamin.—In the treatment of syphilis in its early stages, arsphenamin and neoarsphenamin are administered after various plans of treatment. The medicaments may be given once a week, twice a week, daily for three doses or daily for six doses, etc., and each plan has been endorsed by various physicians so that great confusion exists regarding the best course to pursue. A large number of factors require consideration among which may be mentioned the questions of toxicity, the stage of the disease, the route of administration, the rate of absorption and elimination of the medicament and the rapidity with which the compound is rendered therapeutically active in the body after administration, granting that Voegtlin and Smith are correct in their belief that arsphenamin and neoarsphenamin are not of themselves trypanocidal or spirocheticidal but first require oxidation in the body.

In order to obtain more information on this subject I have infected rats with 500,000 *Tr. equiperdum* by intraperitoneal injection and then subjected them to treatment after various plans, beginning 24 hours after inoculation. Similar experiments have been conducted with rabbits infected with *Spirochaeta pallida*, which will be described in Chapter XXIII. These experiments have shown that considerably higher trypanocidal activity of arsphenamin over neoarsphenamin in trypanosomiasis of the rat when treatment is given in fractions of the single minimal curative dose of each compound; also the advantage of daily treatment with small doses over the one week plan with large doses. The significance of these results in relation to the treatment of syphilis will be discussed in Chapter XXIX.

Toxicity and Trypanocidal Activity of Tryparsamid.—One of the most interesting and highly trypanocidal of the pentavalent arsenical compounds is the sodium salt of N-phenylglycineamid-p-arsonic acid, prepared by Jacobs and Heidelberger[38] in 1919, during the course of the synthesis of new types of arsenic compounds for the treatment of experimental trypanosome and spirochete infections. They were lead to work with the pentavalent arsenicals as presented by the arsonic acids, because of the opportunity

afforded for a study of the relationship between chemical structure and biological action and because such compounds offered the advantage of forming soluble and stable salts and fewer of the preparative uncertainties that have been associated with the usual arseno trivalent compounds.

Phenylglycin-p-arsonic acid, and its homologue, O-methylphenylglycin-p-arsonic acid had been previously described in a German patent and served as a source of the arsenophenyl glycins by reduction; by new methods Jacobs and Heidelberger rendered possible the preparation of a new series of aromatic arsenic compounds in which the free carboxyl group of the glycins was changed to the amid or substituted amid group. N-phenylglycineamid-p-arsonic acid readily yields a colorless, crystalline sodium salt called "tryparsamid" which is extremely soluble in water and forms neutral solutions which are perfectly stable. Brown and Pearce[39] have thoroughly studied the toxicity, trypanocidal and spirocheticidal properties of the sodium salt. In the trypanocidal tests strains of *Tr. brucei*, *Tr. gambiensi*, *Tr. equinum*, *Tr. equiperdum* and *Tr. evansi* were employed in mice, rats, guinea-pigs and rabbits. As a general rule the mice and rats were treated about eighteen to twenty-four hours after infection, guinea-pigs about one week and rabbits about ten to fifteen days after infection, when trypanosomes had been demonstrated in the peripheral blood; the treated animals were kept under observation from one to three months. The results have shown that the different species of trypanosomes varied considerably in their resistance to tryparsamid; the species of animal also had some influence upon the results. The curative dose of 0.2 to 0.3 gm. per kilo of body weight was quite constant for *Tr. brucei*, *Tr. gambiense*, and *Tr. equinum* infections in mice and *Tr. bruce* and *Tr. gambiensi* infections in rats, but with *Tr. equiperdum* and *Tr. evansi* infections in mice, the dose required to effect a cure was considerably higher. The highest dose used, 0.5 gm. failed to cure all mice infected with *Tr. equiperdum*, although there were cures at doses as low as 0.225 and 0.25 gm. With infections of *Tr. evansi*, mice treated with 0.75 gm. did not relapse, but below this dose, cures were uncertain. Both these infections however, were more easily handled in rats than in mice, since there were no relapses with doses of 0.4 gm. in *Tr. equiperdum* and none with 0.3 gm. in *Tr. evansi* infections.

In my tests with *Tr. equiperdum* infections of rats the minimal curative dose of tryparsamid was 0.200 gm. per kilo by intravenous injection; the maximal tolerated dose was 1.0 gm. per kilo. These results have yielded, therefore, the following chemotherapeutic index:

$$\frac{\text{Maximum tolerated dose: 1.0 gm. intravenously}}{\text{Minimal curative dose: 0.2 gm. intravenously}} = 5$$

With the Hygienic Laboratory method of Voegtlin and Smith, I found the minimal effective dose for rats to be 0.3 gm. per kilo and the chemotherapeutic index as follows:

$$\frac{\text{Maximum tolerated dose: 1.0 gm. intravenously}}{\text{Minimal effective dose: 0.3 gm. intravenously}} = 3.3$$

The rapidity of the trypanocidal action of tryparsamid in trypanosomiasis of mice, rats and guinea-pigs is quite marked, the peripheral blood being cleared of organisms in eighteen to twenty-four hours after the treatment of twenty-four hour infections. With rabbits treated after clinical signs of the diseases had been established, a system of repeated dose therapy has been employed by Brown and Pearce with advantage in the treatment of both initial and relapsed infections, especially in those cases in which there is

induration or even necrosis of tissues with weakness and emaciation of the rabbit. The factor of time of repetition or the spacing of doses was in their experience as important as that of size of the dose employed and depends upon the rate, degree, and duration of action of the particular dose of the drug in question. Tryparsamid apparently possesses the power of tissue penetration to a marked degree and the repetition of small doses, such as 0.15 gm. per kilo of body weight on successive or alternate days, has given successful results as regards both the immediate regression and healing of lesions and ultimate permanent cures in severe, chronic infections. In *Tr. brucei* infections intramuscular injections of single or repeated doses yielded practically the same effects as by intravenous injection, while the effects were somewhat slower after subcutaneous injections.

Toxicity and Trypanocidal Activity of Other Organic Compounds of Arsenic.—The results of toxicity and trypanocidal tests with various other trivalent and pentavalent compounds of arsenic including those already discussed, are summarized in Tables 1 and 2. Some of these compounds are of special interest by reason of being intermediate compounds in the preparation of arsphenamin (salvarsan) as the pentavalent compounds atoxyl, the monosodium salt of 4-hydroxyphenylarsonic acid and the sodium salt of 3-nitro-4-hydroxyphenylarsonic acid. The "oxids" of the trivalent arseno compounds are likewise of special interest in relation to the theory of Voegtlin and Smith that the trypanocidal activity of this class of arsenical compounds depends upon oxidation processes in the body.

It is true that the results observed by different investigators with presumably the same compounds have varied, but in this connection it is to be remembered that the results vary considerably according to the technic employed for trypanocidal tests; furthermore, the chemical constitution of many of these substances is so complicated and the methods for their preparation so apt to vary in some detail in different laboratories, that the results should be accepted and evaluated only in broad and general manner.

Mechanism of Trypanocidal Activity of Arsenical Compounds.—Since atoxyl is practically without trypanocidal activity in the test tube Ehrlich has maintained that it must be reduced from its comparatively inactive in pentavalent form to the more active trivalent form before parasiticidal effects become apparent. Breinl and Nierenstein[40] stated in 1909 that in their opinion atoxyl when administered first combined with the serum proteins by means of the amino group, followed by a process of oxidation of the benzene nucleus by means of oxidases from the tissues as well as probably from the trypanosomes themselves with the liberation of arsenic. At the same time a process of reduction takes place by which atoxyl is split into arsenious acid and anilin, the latter being eliminated in the feces. The arsenic liberated by this dual process of oxidation and reduction is regarded as being actively trypanocidal in its nascent state. Later Nierenstein[41] modified this theory somewhat to state that not only was arsenic rendered trypanocidal in this manner, but that the amino group in atoxyl as well as in certain dyes, was directly trypanocidal.

Levaditi's "Trypanotoxyl" Theory.—Levaditi[42] has stated that atoxyl is not primarily trypanocidal *in vivo* but first unites with substances in the liver with the production of a trypanocidal substance designated as "trypanotoxyl." In other words it would appear that the trypanocidal and probably the spirocheticidal activity of the organic arsenical preparations *in vivo* is not a direct and simple chemical action of these substances upon the parasites but that a change occurs in the blood and other tissues. This change may be: (a) the formation of new combination compounds presumably with the

TABLE 1.—TOXICITY AND TRYPANOCIDAL ACTIVITY OF TRIVALENT ORGANIC ARSENICAL COMPOUNDS

SODIUM SALTS OF	PER CENT. ARSENIC	TEST ANIMAL	ROUTE OF ADMINISTRATION	TOXICITY PER KILO		TRYPANOCIDAL TESTS	
				DOSIS LETHALIS	DOSIS TOLERATA	ORGANISM	DOSE PER KILO
Arsenious acid	45.6	Rat	Intravenous	0.0075	.....	Tr. equiperdum	0.0075
Methyl arsenious acid	70.7	Rat	Intravenous	3.75*	.....	Tr. equiperdum	3.75*
Ethyl arsenious acid	59.0	Rat	Intravenous	5.0*	.....	Tr. equiperdum	2.25*
p-arsenophenylglycine	35.2	Rat	Intravenous	0.169	.....	Tr. equiperdum	0.010
p-arsenophenylglycine	....	Mouse	Subcutaneous	0.250	.....	Tr. brucei	0.200
p-arsenophenylglycine	....	Mouse	Subcutaneous	0.600	.....	Tr. brucei	0.200
p-arsenophenylglycine	....	Rat	Subcutaneous	0.400	.....		
p-arsenophenylglycine	....	Guinea pig	Intraperitoneal	0.120	.....	Tr. brucei	0.080
p-arsenophenylglycine	....	Rabbit	Intravenous	0.220	.....	Tr. brucei	0.125
Triphenyl arsin	25.3	Rat	Intravenous	>20.0*	.....	Tr. equiperdum	>20.0*
Phenylarsenoxid	44.6	Rat	Intravenous	0.0063	.....	Tr. equiperdum	0.0013
Diphenyl arsenious oxid	31.5	Rat	Intravenous	7.5*	.....	Tr. equiperdum	5.0*
p-oxy-m-aminophenylarsenoxid (arsenoxid)	27.3	Rat	Intravenous	0.02	.....	Tr. equiperdum	0.0015
3-amino-4-hydroxyphenylarsinoxid (arsenoxid)	37.7	Rat	Intravenous	.....	0.048	Tr. equiperdum	0.006
3-amino-4-hydroxyphenyl arsin	40.5	Rat	Intravenous	.....	0.042	Tr. equiperdum	0.0047
3, 3-tetra-amino-4, 4-dihydro-oxy-arsenobenzene	37.9	Rat	Intravenous	.....	0.075	Tr. equiperdum	0.013
3, 3-tetra-acetyl-tetra-amino-4, 4-dihydro-oxy-arsenobenzene	26.6	Rat	Intravenous	.....	0.150	Tr. equiperdum	0.060
3, 3-diacetyl-diamino-4 , 4-dihydro-oxy-arsenobenzene	33.3	Rat	Intravenous	.....	0.136	Tr equiperdum	0.014
4, 4-dihydro-oxy-arsenobenzene-3, 3-diamino-acetic acid	31.1	Rat	Intravenous	.....	0.150	Tr. equiperdum	0.009
Arsphenamin (salvarsan)	31.1	Rat	Intravenous	.....	0.120	Tr. equiperdum	0.002
Necarsphenamin (neosalvarsan)	19.0	Rat	Intravenous	.....	0.300	Tr. equiperdum	0.004

* Expressed in terms of cubic centimeters of 1:100 arsenic equivalent solution per kilo body weight (Voegtlin and Smith).

TABLE 2.—TOXICITY AND TRYPANOCIDAL ACTIVITY OF PENTAVALENT ORGANIC ARSENICAL COMPOUNDS

SODIUM SALTS OF	PER CENT. ARSENIC	TEST ANIMAL	ROUTE OF ADMINISTRATION	TOXICITY PER KILO		TRYPANOCIDAL TESTS	
				DOSIS LETHALIS	DOSIS TOLERATA	ORGANISM	DOSE PER KILO
Cacodylic acid (sodium cacodylate)	33.3	Rat	Intravenous	1000.0*	.....	Tr. equiperdum	1000.0*
4-aminophenyl arsonic acid (arsanilic acid; atoxyl)	31.4	Rat	Intravenous	.....	0.150	Tr. equiperdum	0.060
Ethyl arsonic acid (mon arsone)	35.3	Rat	Intravenous	>200.0*	.....	Tr. equiperdum	>200.0*
Methyl arsonic acid (arrhenal)	25.68	Mouse	Subcutaneous	2.5	.....	Tr. brucei	0.2 to 0.5
Arsenic acid	31.3	Rat	Intravenous	0.0075	.....	Tr. equiperdum	0.0075
Phenylglycine-p-arsonic acid	27.17	Rat	Intravenous	2.06	.....	Tr. equiperdum	1.03
N-phenylglycine-amide-arsonic acid	24.3	Rat	Intravenous	2.05	.....	Tr. equiperdum	0.205
N-phenylglycine-amide-arsonic acid	.....	Mouse	Subcutaneous	2.5	.....	Tr. brucei	1.0
N-phenylglycine-amide-arsonic acid	.....	Rat	Intraperit.	1.2	.....	Tr. brucei	0.7
N-phenylglycine amid-p-arsonic acid	25	Mouse	Intraperit.	2.25	.....	6 strains	0.2 to 0.5
N-phenylglycine amid-p-arsonic acid	.....	Rat	Intraperit.	0.75	.....	6 strains	0.2–0.4
N-phenylglycine amid-p-arsonic acid	.....	Guinea pig	Intraperit.	1.5	.....	6 strains	0.15–0.25
N-phenylglycine amid-p-arsonic acid	.....	Rabbit	Intravenous	0.75 to 0.9	.....	6 strains	0.2 to 0.5
4-acetyl-amino-phenyl arsonic acid (arsacetin)	21.1	Rat	Intraven.	2.10	.....	Tr. equiperdum	0.105
3-nitro-4-hydroxyphenyl arsonic acid	28.5	Rat	Intravenous	.....	0.096	Tr. equiperdum	0.09
3-nitro-4-hydroxyphenyl arsonic acid	32.19	Rat	Intravenous	.....	0.500	Tr. equiperdum	0.08

* Expressed in terms of cubic centimeters of 1:100 arsenic equivalent solution per kilo of body weight (Voegtlin and Smith).

proteins; (b) a simple chemical change of oxidation or reduction without the formation of a combination product; (c) a production of antibodies, or (d) a combination of one or more of these factors.

The liver cells however, do not appear in my experiments to increase the trypanocidal activity or toxicity of arsphenamin to a greater extent than physiological saline solutions. I have prepared 2 per cent. alkalinized solutions of arsphenamin in 20 per cent. suspensions of very finely divided rabbit liver and after placing them in an incubator for an hour have determined the toxicity and trypanocidal activity at the same time similar solutions of the same products were prepared in saline solutions and incubated one hour before injection; solutions were likewise tested immediately after preparation. The solutions in saline solution and liver emulsion after standing one hour in an incubator were usually, but not always, slightly more toxic and more trypanocidal than freshly prepared solutions. As a general rule however, the liver cell solutions were slightly less toxic than the saline solution preparations although the trypanocidal properties were about equal. These effects were ascribed to the production of oxidation products of arsphenamin as the result of standing in an incubator for an hour before injection. It is possible that enzymes from the liver cells may hasten the process, but there were no evidences of the formation of therapeutically active combination compounds of the arsphenamin and cellular substances.

We have already noted that solutions of arsphenamin and neoarsphenamin exposed to air acquire increased trypanocidal activity presumably by the formation of oxids. In this connection it may be recalled that Swift and Ellis found that heating serum taken from blood drawn within an hour after the intravenous injection of arsphenamin, increased its spirocheticidal properties probably by the same process. A question of importance is whether this change is brought about more rapidly in serum or emulsions of organs and particularly of the liver, than in water or saline solution. I have conducted experiments along these lines which tend to show that the menstrum has but slight influence upon the process. Leaving the solutions stand for 2 hours at room temperature increased the rate of trypanocidal activity but as stated above, the trypanocidal activities of the solutions in saline solution were about the same as solutions in rat serum, blood and suspensions of liver, spleen and brain. In other words, a chemical change had occurred tending to increase trypanocidal activity, but this was not appreciably influenced by the menstrum.

Voegtlin and Smith's Oxidation and Reduction Theory.—It is clearly established that trivalent compounds of arsenic are more toxic and likewise more trypanoicidal than pentavalent compounds. The same is true of compounds of antimony and some other metals. As shown by the work of Voegtlin and Smith the time required for the appearance of toxic symptoms or evidences of trypanocidal activity is generally longer for pentavalent than for trivalent arsenical compounds and since Binz and Schultz (1879) and others have shown tha tarsenic is reduced in the body, it is reasonable to assume that the longer latent period required by the pentavalent compounds is for the reduction of a sufficient amount of the drug to the trivalent form.

Voegtlin and Smith have furthermore shown that compounds containing arsenic in the "oxid" form (R.As = O) exert toxic and trypanocidal effects much more rapidly than arsenobenzol compounds. Their method is splendidly adapted for such studies on the rate of disappearance of trypanosomes from the blood and with it they have clearly demonstrated that after the intravenous injection of the minimal effective dose of a compound belonging to the arsenoxid group, the number of trypanosomes at once decrease until

all have been killed, requiring ten to sixty minutes, depending on the size of the infection. The immediate onset of the reaction and the great regularity by which it proceeds, shows in their opinion, that the compounds of the arsenoxid type are directly trypanocidal.

On the other hand according to the experiments of Voegtlin and Smith, the injection of twice or even several times the minimal effective dose of the arsenobenzol derivatives and the pentavalent arsenicals does not produce material trypanocidal effects for a period of one to six hours and in their opinion, these compounds are not themselves trypanocidal, but must be first converted into the active "oxids," the oxidation in the case of the arsenobenzol derivatives being as follows:

$$R.As = As.R \rightarrow R.As = O.$$

If this view is correct and I believe that Voegtlin and Smith have made out a strong case for it although Schumacher[42a] has recently advanced the hypothesis that arsphenamin destroys spirochetes by abstracting oxygen from them in the tissues, arsphenamin and neoarsphenamin are not themselves trypanocidal but first require oxidation in the body to the therapeutically active "oxids." Of course in the preparation of the solutions sufficient oxids may be produced or be present in the powder, to exert some immediate effects, but their demonstration of the latent period following the injection of arsenicals belonging to the arsenobenzol group and pentavalent compounds before the main trypanocidal effects are apparent, naturally leads to the conclusion that some change is required in the body and it is natural to surmise that the oxids are important since the first logical step in oxidation would be the breaking down of the arseno-grouping to the oxid grouping. In addition to their own experiments Voegtlin and Smith draw attention to other facts in support of their theory. For example, the greater toxicity and trypanocidal activity of solutions of arsphenamin and neoarsphenamin kept in contact with air is attributed to the effects of oxidation. As first shown by Ehrlich and his associates, this leads to the production of para-oxy-m-amino-phenyl-arsenoxid which is seven to ten times more toxic than arsphenamin and neoarsphenamin and likewise more trypanocidal; furthermore, as shown by Voegtlin and Smith it exerts a direct action on the parasites as indicated by the absence of a latent period after intravenous injection. In our own experiments, previously discussed, solutions of arsphenamin and neoarsphenamin, shaken in air or left in contact with air, also showed a tendency for becoming more toxic and more trypanocidal unless the process of oxidation was greatly prolonged when a decrease of toxicity and trypanocidal activity followed apparently due to the formation of the pentavalent amino-hydroxy-phenyl arsonic acid, first produced by Ehrlich and Bertheim in 1912 by oxidizing arsphenamin in alkaline solution by means of hydrogen peroxid.

The fact that arsphenamin and neoarsphenamin are less trypanocidal and spirocheticidal in the test tube than in the living body is also interpreted as evidence in favor of this theory of oxidation. Likewise observations showing that pentavalent arsenical and antimonial compounds are relatively inert for bacteria, yeast and protozoa *in vitro*. As previously stated Ehrlich advanced the theory that the pentavalent arsenicals must be reduced in the body before becoming therapeutically active, but reduction also leads to the same As:O-grouping as is formed on the oxidation of the arsenobenzol grouping. Since, however, reduction is probably less easily accomplished than oxidation of the arsenobenzols, it may be expected that the latent period would be longer before trypanocidal effects would be apparent after intravenous injection, and this has actually been found the case by Voegtlin and Smith. By

reason of this longer latent period these investigators have very properly pointed out that the dose of those compounds which undergo reduction before becoming therapeutically active must be larger than those readily reduced and oxidized, because while these processes are going on, a certain amount of the compound is being excreted or fixed in the tissues. Since arsphenamin is eliminated much more slowly than neoarsphenamin it is probable that the arsphenamin fixed by the tissues is oxidized to the parasitocidal oxid much more slowly than neoarsphenamin which is more rapidly oxidized and eliminated. I will, however, later discuss these questions in more detail in relation to the absorption and elimination of arsphenamin and neoarsphenamin and the bearing these have in addition to the question of rapidity of oxidization, upon the treatment of syphilis with special reference to the choice of compounds in the treatment of the acute *versus* chronic stages of this disease.

Theory of Antibody Production.—While the trypanocidal activity of the pentavalent and trivalent arsenical compounds appears to depend upon the production *in vivo* of new compounds by processes of oxidation and reduction, the question arises whether curative activity is aided by the production of antibodies. Not infrequently one hears that the curative activity of mercury and the arsenical compounds in syphilis is due to the fact that these substances stimulate the body cells to produce antibodies for *Sp. pallida* and that the same processes may be operative in the cure of trypanosomiasis.

As stated in Chapter XI there is evidence to show that a temporary period of immunity follows the cure of trypanosomiasis of mice treated with various dyes. This is also true after cures effected by atoxyl, arsacetin and arsenophenylglycin although this immunity is of very brief duration following cures of *Tr. equiperdum* infections of rats with arsphenamin and neoarsphenamin.

The question naturally arises, is the cure of trypanosomiasis by a single large dose of an arsenical, antimonyl or other trypanocidal compound aided by the production of antibodies or is it a matter primarily and solely of chemical destruction by products of the drugs? The possible role of curative immunity in chronic infections is a different matter because time is afforded for the production of immune principles. What are the chances of immunological cures in acute infections of mice and rats?

Uhlenhuth and Woithe[43] stated in 1908 that atoxyl probably owed some of its trypanocidal activity to an influence upon antibody production but were unable to produce experimental evidence in favor of the theory. Terry[44] found that the administration of arsacetin prolonged the excretion of dichlorbenzidin plus amidonaphtol disulphonic acid 1.8.3.6 and thereby aided the immunity engendered by this substance in mice infected with *Tr. evansi.* Weisbach[45] claimed that the administration of salvarsan results in an increase of agglutinins and complement; Ciuca[46] observed that the injection of tartar emetic and salvarsan was followed by an increase of serum complement in normal and trypanosome-infected animals, while the administration of atoxyl caused a decrease of complement in the area of normal animals and in a proportion of trypanosome infected animals; Toyama and I[47] observed that while massive doses of arsphenamin and mercuric chlorid tended to decrease the amount of complement in the serum of rabbits, smaller doses tended to augment the complement content after a primary decrease, in both human subjects and rabbits.

However, the evidence in favor of the theory that the arsenical compounds owe part of their curative activity in trypanosomiasis to an influence upon antibody production is very meager and inconclusive and during the past two years I have sought to gain additional information on this subject.

In the first place, experiments with rats have shown that the preliminary injection of single and repeated doses of alkalinized solutions of arsphenamin in amounts varying from 0.01 to 0.02 gm. per kilogram of weight, had no influence upon increasing the resistance of the rats to infection with *Tr. equiperdum* injected intraperitoneally at intervals of three to sixteen days after the last injection of the drug; that is, the medicament in these experiments did not show any evidences at all of increasing the natural resistance or immunity of these animals either by antibody production or other immunological agencies. Similar results of a negative character were observed with neoarsphenamin administered in doses varying from 0.02 to 0.03 gm. per kilogram.

Vaccines of *Tr. equiperdum* were prepared and employed for the immunization of rabbits by intravenous injection of ascending doses arranged according to body weight. At the same time some of these animals received intravenous injections of alkalinized solutions of arsphenamin in dose of 0.01 gm. per kilo and solutions of neoarsphenamin in dose of 0.02 gm. per kilo four hours after each injection of vaccine. At frequent intervals the serum of each animal was examined quantitatively for agglutinins, complement fixing antibodies (alexo-fixagins) and trypanolysins.

It was noted that the rabbit reacts poorly and tardily with the production of these antibodies for *Tr. equiperdum* even when a large number of heat killed trypanosomes have been given along the same general lines as employed for the production of immune bacterial agglutinins, hemolysins, etc., in these animals. Animals receiving the injections of arsphenamin and neoarsphenamin at the same time did not produce any more of these antibodies than animals receiving only the vaccine.

These results have shown that arsphenamin and neoarsphenamin at least do not have any appreciable influence upon increasing specific antibodies in rabbits immunized with *dead* trypanosomes; antibodies are produced but not more so in arsphenamin and neoarsphenamin treated animals than in the untreated controls.

It is of course difficult to measure the degree of immunity by test tube experiments and the chances of error are considerable, but I believe that antibody production in trypanosomiasis is seldom high and develops but slowly. Both the degree and rapidity of the immunity may be influenced by the administration of trypanocidal agents, the drug releasing antigenic principles from the parasites rather than stimulating the antibody producing tissues. The different compounds vary however, in this activity. Furthermore, these compounds act better with living trypanosomes in the production of this immunity than with vaccines of dead organisms, but I very much doubt if immunity can be produced quickly enough to aid in the cure of an acute trypanosome infection of the mouse and rat when sterilization is brought about by a powerful trypanocidal drug in a matter of sixteen to twenty-four hours.

In the treatment of chronic infections of guinea-pigs and rabbits or of acute infections of rats with repeated doses of a trypanocidal drug, immunity may aid in holding in check the infection and even in bringing about a cure, but in acute infections, this does not appear to be the case.

Route of Administration of Arsenicals in Relation to Toxicity and Trypanocidal Activity.—As will be discussed with more detail in Chapters XVIII and XIX, the route of administration of the arsenicals is a subject of considerable practical importance in the chemotherapy of trypanosomiasis and syphilis. Ehrlich found quite early in his work with salvarsan that intramuscular injections were more effectual in the treatment of experimental trypanoso-

miasis and spirochaetosis than intravenous injections and some clinicians are convinced that this is true in the treatment of syphilis of human beings with arsphenamin and its substitutes. The toxicity of arsphenamin and neoarsphenamin for mice and rats is influenced by the route of administration and in the treatment of trypanosomiasis of the rat it has been observed that intramuscular injections of arsphenamin and neoarsphenamin are just as effective as intravenous injection; indeed, intramuscular injections of arsphenamin are apt to be slightly more effective than intravenous injections, as shown by Voegtlin and Smith[48], and I have found the same to be true of sulpharsphenamin. There is therefore, a great and urgent need for efficient arsenicals capable of being injected subcutaneously or intramuscularly with little or no local reaction and especially for the treatment of syphilis and trypanosomiasis of human beings, not only because this route of administration is technically easier than intravenous injections, but because the therapeutic results are likely to be better and especially for the treatment of chronic infections when slower elimination is required. Sulpharsphenamin and the new combination compounds of arsenic and bismuth are compounds of this highly desirable class.

Arsenic Resistance of Trypanosomes.—Arsenic resistance or fastness of trypanosomes is an acquired state whereby the parasites are enabled to withstand several times the dose of arsenical which originally would have destroyed them. As stated in Chapter I the phenomenon was discovered more or less accidentally by Ehrlich working with mice infected with trypanosomes and treated with arsacetin and arsenophenylglycin. Since then a great deal of work has been done on the subject of drug fastness in general and particularly with the arsenicals, tetrazo dyes (trypan-red and trypan-blue) and triphenylmethan dyes (parafuchsin) with trypanosomes, spirochetes and bacteria with the general result that acquired resistance or tolerance of parasites for drugs and poisons is known to be a general biological phenomenon observed with a multitude of chemicals and various organisms.

Browning[49] succeeded in rendering *Tr. brucei* resistant to atoxyl and arsacetin by feeding these compounds or injecting them subcutaneously in sub-curative doses. Naturally great interest attaches to the question whether or not *Tr. gambiensi* and other trypanosomes can acquire resistance to arsphenamin and neoarsphenamin as bearing a very practical relation to the treatment of African sleeping sickness as well as in relation to the treatment of syphilis. Experiments have shown that *Tr. gambiensi* and *Tr. equiperdum* do acquire resistance to both of these compounds. Voegtlin and Smith have stated that *Tr. equiperdum* injected into rats which two weeks previously had received 0.1 to 0.2 gm. of arsphenamin and neoarsphenamin respectively per kilogram of weight, acquired a resistance. I have had a similar experience on three different occasions during the past eight years. The comparative ease with which trypanosomes can be made resistant to arsphenamin, neoarsphenamin and the arsenical compounds in general, renders imperative the greatest care in laboratories carrying strains of different trypanosomes; otherwise a resistant strain may gain circulation and lead to very erroneous results and deductions in chemotherapeutic investigations. Possibly something like this happened to the strain of *Tr. equinum* reported by Halberstadter[50] as *naturally* resistant to atoxyl, arsacetin, arsenophenylglycin and salvarsan *in vitro* and *in vivo*. His strain had never been subjected to the influence of these compounds as far as he knew, but I know from experience that it is easy to be mistaken. This strain was fully susceptible to tartar emetic although Morgenroth and Halberstadter[51] found a strain rendered resistant to arsacetin likewise resistant to antimony. This arsacetin-fast

strain was likewise resistant to salvarsan and to a lesser degree to arsenophenylglycin. After discontinuing treatments, resistance to tartar emetic was lost while resistance to the arsenical compounds persisted.

Of great interest in this connection is whether trypanosomes rendered resistant to arsphenamin are likewise resistant to neoarsphenamin and the reverse. Browning found that an atoxyl resistant strain of *Tr. brucei* was resistance to arsacetin and one other derivative of atoxyl. In the treatment of trypanosomiasis therefore, probably no advantage is to be gained by "switching" from one of these compounds to the other to overcome possible resistance of *Tr. gambiensi* or other pathogenic trypanosomes. However, with less closely related arsenical compounds this group resistance is not so much in evidence. For example, the arsphenamin and neoarsphenamin resistant strains of *Tr. equiperdum* in my laboratory were susceptible to atoxyl and tryparsamid. In practice, therefore, when it is suspected that the trypanosomes have acquired as resistance to any particular medicament it is well to "switch over" to some other trypanocidal agent as far removed in chemical relationship as possible.

Curiously, as shown by Lake and Probey,[52] arsenic resistant strains may be proportionately less resistant to neoarsphenamin than to arsphenamin; for example, they found that a neoarsphenamin which was only about one-third as effective as arsphenamin with a "normal" strain was fully as effective as arsphenamin with several "arsenic-fast" strains. Voegtlin and his associates confirmed this observation but the differences were not as great. The latter also found that arsenic resistance and virulence are not related in any way and that while Mesnil and Beaumont[53] were unable to change the resistance of a strain resistant to atoxyl in mice by passing it through 43 rats, yet the resistance of their strain to arsphenamin in rats was greatly reduced by passage through rabbits and a dog, the decrease gradually disappearing however, as a result of repeated transfers in normal rats. Voegtlin and his associates also showed that if a rat is inoculated with a mixture of normal and arsenic-fast strains, the administration of an ordinary sterilizing dose of arsenoxid like 0.5 c.c. M/100 solution per kilogram of body weight, will enable one to recover the arsenic-fast strain since it resists destruction. I have accomplished the same result by administering arsphenamin in dose of 0.003 gm. per kilogram twenty-four hours after inoculation with a mixture of normal and arsenic resistant strains, the latter requiring a dose of approximately 0.015 gm. for sterilization while the normal strain was regularly killed off *completely* by 0.003 to 0.004 gm. per kilogram.

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CHAPTER XIII

TOXICITY AND TRYPANOCIDAL ACTIVITY OF COMPOUNDS OF ANTIMONY

Next to organic compounds of arsenic, compounds of antimony have proven most trypanocidal, not only in the chemotherapy of experimental trypanosomiasis but likewise in the treatment of trypanosome diseases of human beings and the lower animals. Tartar emetic and other antimonyls have been also employed in the treatmemnt of various other protozoal and metazoal diseases so that the group is of much interest and importance in the chemotherapy of disease.

Trypanocidal Activity of the Antimony Tartrates.—In 1908, Plimmer and Thompson[1] first discovered the trypanocidal activity of potassium antimony tartrate and sodium antimony tartrate in rats infected with *Tr. brucei* and *Tr. evansi.* A dose of 1 c.c. of a 1-per cent. solution of the potassium salt was usually toxic in twenty-four to forty-eight hours but likewise brought about a rapid disappearance of the trypanosomes. The sodium

salt in dose of 0.35 to 0.5 c.c. of 1 per cent. solution was less toxic and caused the disappearance of trypanosomes from the peripheral blood within half an hour even when it was "swarming with the parasites." Relapses, however, sometimes occured. Plimmer and his associates continued their studies on the effect of various antimonials in rats and dogs and in conjunction with Bateman, made important experiments to determine where the trypanosomes rest during the period in which the peripheral blood is free from them after treatment with antimony, with the result that in rats trypanosomes were found to live longest in the bone marrow and liver.

At the same time and independently Mesnil and Brimont[2] made extensive studies with the double tartrate of antimony and potassium (tartar emetic) and found that its trypanocidal activity varied considerably with different strains of trypanosomes. Thus *Tr. evansi* (surra) infections of mice were generally cured by one injection of 0.3 to 0.35 mgm. per 20 grams of weight; when relapses occurred cures were effected by additional treatments. One strain of surra (*mauritius*) employed for infecting 25 mice was atoxyl-fast but was readily influenced by tartar emetic since 21 of the mice were cured. Similar results were observed in the treatment of mice infected with *Tr. equiperdum* (dourine). With mice infected with *Tr. brucei* (nagana) and *Tr. equinum* (caderas), however, the results were not so good; following an injection, the trypanosomes usually disappeared for a while but relapses occurred continually and most of the mice succumbed. In *Tr. gambiensi* infections of rats the injection of 1 mgm. per 10 grams of weight usually brought about a temporary disappearance of trypanosomes, but relapses generally occurred in four to sixteen days; the animals gradually lost weight and finally succumbed if not to trypanosomiasis, then to the effects of repeated injections of the medicament. Boyce and Breinl[3] were less successful with sodium antimony tartrate in the treatment of rats infected with *Tr. equiperdum* and stated that the local effects were very severe, causing necrosis and sloughing. Tsuzuchi[4] found tartar emetic highly trypanocidal and particularly when employed with arsenical compounds in a combination therapy.

Trypanocidal Activity of Various New Compounds of Antimony.—Breinl and Nierenstein continuing this work, prepared *p*, *m*, and *o* amino-phenyl-stibinic acids analogous to atoxyl. These new compounds were administered in the form of sodium salts to rats, dogs and monkeys infected with *Tr. brucei*, *Tr. evansi* and *Tr. gambiensi* and the *p*-compound was found to possess a fairly powerful trypanocidal action, although not as rapid in action as sodium antimonyl tartrate.

Thompson and Cushing[5] examined a number of antimony compounds in rats infected with *Tr. brucei*. Among those that were inefficient or valueless for one reason or another were diphenylstibinichlorid, potassium meta-antimoniate, colloidal antimony oxid and a glycerid of antimony. Among the best compounds were the combinations of antimony with oxyacids of the fatty series, as the lactate, citrate, malate and mucate salts. The potassium and sodium salts of the malates and tartrates were equally trypanocidal, but it was thought that the substitution of an alkyl radicle for the potassium or sodium of the salts seemed to have some advantage. Best results were observed with solutions of ethylantimonyl tartrate, prepared by heating freshly precipitated antimony oxid with ethyl tartrate to about 150°C. in sealed tubes. A combination therapy of atoxyl and ethylantimony tartrate did not yield appreciably better results than the latter alone, the mixtures being more toxic and scarcely more trypanocidal.

The next notable contribution to this subject was made by Rountree and Abel.[6] Three new compounds were prepared, the triamid of antimony thioglycollic acid, a new antimony-thioglycollic ester and sodium antimony thioglycollate. The ester, however, had such a low solubility in water that it was not regarded as suitable for therapeutic tests. Trypanocidal tests with the remaining two compounds were conducted with rats, dogs, rabbits and a donkey employing *Tr. brucei*, *Tr. evansi* and *Tr. equiperdum*. Both appeared to possess equal or possible slightly greater trypanocidal activity than tartar emetic and possessed the advantage of being better adapted for subcutaneous injection. Most interest was shown in the antimonylthioglycollate, in solutions of which the antimony occurs as a complex organic ion.

Uhlenhuth, Mülzer and Hügel[7] found a colloidal preparation of antimony effective in repeated doses, but most of their work was carried out with hen spirillosis and rabbit syphilis, to which I will make further reference in Chapter XXVII. Kolle, Hartock and Rothermundt[8] observed particularly good results in the treatment of mice infected with *Tr. brucei* with organic and inorganic compounds containing antimony in trivalent form. Antimony trioxid ("trixidin") in 30 per cent. suspension in oil was particularly non toxic upon intramuscular injection and was stated to yield a chemotherapeutic index of 1:100. Metallic antimony in oil suspension was likewise effective as also tartar emetic, dimethylphenylpyrazalon, dimethylphenylpyrazalonacethyl antimony trichlorid, etc. Kolle and his associates also showed at this time that it is possible to influence and even cure as many as 66 per cent. of mice, rats, guinea-pigs, rabbits and monkeys infected with *Tr. equiperdum*, *Tr. brucei* and *Tr. gambiensi* by inunctions of ointment containing metallic antimony. The establishment of "depots" of the medicament by cutaneous application or intramuscular injection results in the drug being more slowly absorbed and eliminated yielding thereby a more prolonged effect. In a subsequent article Kolle, Hartock and Shürman reported additional experiments with antimony trioxid ("trixidin"). Intramuscular injections in rabbits, dogs and monkeys produced abscesses, but therapeutic success followed the intravenous injection of particularly fine suspensions in saline solution prepared from tartar emetic, in the treatment of donkeys infected with *Tr. brucei*, dogs and guinea-pigs infected with *Tr. equiperdum* and rabbits infected with *Tr. brucei* and *Tr. equiperdum*. Multiple doses were usually required. Hoffman[9] confirmed this work with antimony trioxid, establishing the toxic dose for rabbits by intravenous injection of suspensions in 20 per cent. glucose, 10 per cent. gum arabic or best of all, in a 7.5 per cent. gum arabic and 20 per cent. glucose solution, as about 0.100 to 0.120 gm. per kilogram of weight and the curative dose 0.045 gm. per kilo for *Tr. brucei* infections and 0.025 to 0.030 gm. per kilo for *Tr. equiperdum* infections. Horst[10] has likewise reported upon the curative activity of antimony trioxid, calling attention however, to the accidents caused by embolism when suspensions are injected intravenously. Tartar emetic was likewise found trypanocidal.

Voegtlin and Smith,[11] have recently renewed interest in the trypanocidal activity of salts of antimony with special reference to antimonyllactate. Especially good effects were noted with antimonythioglycollate, first used by Rountree and Abel as discussed above, tartar emetic and antimonyllactate. White rats and *Tr. equiperdum* were employed, the trypanocidal tests being conducted by their method and yielding chemotherapeutic indices varying from 3 to 6. In trypanocidal tests after my own method, tartar emetic yielded an index of 3.

Fargher and Gray[12] have recently prepared a series of compounds of antimony and tested their toxicity upon mice by intravenous injections; trypanocidal tests were not conducted and it is not possible therefore, to express an opinion of their comparative chemotherapeutic values. These compounds comprised the salts of the alkali metals, cinchona alkaloids, anilin and other substances. The percentage of antimony varied from 46.56 in a partially polymerized mixture of sodium phenylstibinate to 17.11 in cinchonine antimonyl tartrate. Toxicity varied from 0.174 gm. per kilogram of weight of sodium m-acetylaminophenylstibinate to 0.010 gm. per kilogram of barium antimonyl tartrate.

Mechanism of Trypanocidal Activity of Antimony Compounds.—The majority of investigators in this field have noted the rapid disappearance of trypanosomes from the blood of infected animals after the administration of some antimony compounds, and especially after intravenous infections. Some antimony compounds appear to exert an immediate toxic effect upon the parasites, the resistance of different species varying, however, in susceptibility to this toxic action. The effects are so rapid in the case of trivalent compounds that there is practically no basis at all for the theory that antimony is not primarily trypanocidal, but speeds up the production of antibodies which are the active trypanocidal agents. Voegtlin and Smith have seen no evidences of agglutination or phagocytosis of trypanosomes in the blood stream preliminary to their disappearance. Apparently they are first rendered incapable of multiplication, followed by death and extremely rapid disappearance from the blood probably largely by cytolysis through the agency of autolytic or blood enzymes.

As shown by Voegtlin and Smith, a pentavalent antimony compound (antimonic potassium tartrate prepared by oxidizing a solution of antimonyl-potassium tartrate with chlorin gas) was of low toxicity for both rat and trypanosomes whereas a trivalent compound (antimonyllactate prepared from lactic acid and antimony trioxid) while more toxic for the host, was likewise more toxic for the trypanosomes. As mentioned above Kolle and his associates have long maintained that the trivalent compounds were more trypanocidal than pentavalent compounds. Voegtlin and Smith have also observed that the toxic effects and trypanocidal activity of the trivalent compound occurred more rapidly than in the case of the pentavalent compound and on the basis of their results advanced the theory that the pentavalent compounds first require reduction to the trivalent form before marked trypanocidal effects may occur; an exactly similar theory has been advanced by these investigators to explain the trypanocidal activity of arsenical compounds as discussed in Chapter XII.

Nothing is known of the influence of immunological factors in relation to the trypanocidal activity of antimonyl compounds. In my experiments after the cure of *Tr. equiperdum* infections of rats with tartar emetic (0.020 gm. per kilogram), a period of immunity of five days follows during which the animals cannot be re-infected. The resistance following this dose of emetic alone in control rats does not usually last longer than 36 hours.

This immunity following cure is analogous to that first observed by Ehrlich and Shiga following the cure of *Tr. brucei* infections of mice with trypan-red discussed in Chapter XI. A similar immunity follows the cure of trypanosomiasis of rats with organic arsenical compounds, as discussed in Chapter XII. I believe that this immunity apparently engendered by antigenic principles released from living trypanosomes by antimonyl compounds, may and probably does, exert a curative activity in the treatment of chronic trypanosomiasis of human beings and the lower animals, but plays

no part at all in the cure of acute infections with single large doses of antimonyl compound.

Acquired Resistance of Trypanosomes to Antimony.—As previously mentioned in preceding chapters it is comparatively easy to render trypanosomes "resistant" or "fast" to the dyes and arsenical compounds. Much less work has been conducted along these lines with the antimonyl compounds.

Halberstadter[13] has reported that a strain of *Tr. equinum* naturally (?) resistant to atoxyl, arsacetin and salvarsan could not be rendered resistant to tartar emetic. Morgenroth and Halberstadter[14] however, were able to render an acquired arsacetin fast strain resistant to tartar emetic by a single treatment. Mesnil and Brimont[15] observed that a strain of *Tr. evansi* which was atoxyl resistant, also developed resistance or tolerance for tartar emetic. With *Tr. brucei*, however, the results were more doubtful. Rountree and Abel were unable to render *Tr. brucei* definitely resistant to sodium antimony thioglycollate, their work in this subject being summed up in the statement that "resistance has not been noted in the prolonged treatment of any single species of animals infected with nagana and that the only phenomena suggesting tolerance have been encountered in the strain which was subjected to treatment in two different hosts, the donkey and the rat." Working with tartar emetic administered in subcurative doses to rats infected with *Tr. equiperdum*, I have succeeded in rendering the parasites appreciably more resistant to this compound after fourteen transfers; the strain, however, had shown no variations in virulence for mice and rats and no change in susceptibility to arsphenamin and neoarsphenamin. I believe, therefore, that trypanosomes can be made antimony-resistant, but not as quickly or readily as they may be rendered arsenic-resistant.

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CHAPTER XIV

TRYPANOCIDAL ACTIVITY OF MERCURIAL COMPOUNDS

Although inorganic and organic compounds of mercury are known to possess spirocheticidal properties and have been employed for hundreds of years in the treatment of syphilis, their trypanocidal activity is very feeble. Even when administered in amounts approximating the maximum tolerated

doses for animals infected with different kinds of trypanosomes, there are no demonstrable trypanocidal effects at all or, at most, a mere retardation of development of trypanosomiasis for a day or two as compared with untreated controls.

Mercurial compounds, however, are not entirely devoid of trypanocidal effects; this can be shown by *in vitro* or better still, by combined *in vitro* and *in vivo* tests to which I will shortly refer. The lack of trypanocidal effects *in vivo* appears to be due in large part to the high toxicity of these compounds preventing the administration of sufficiently large doses. They do not, however, possess anywhere near the trypanocidal activity of the arsenobenzols; arsphenamin, for example, is frequently trypanocidal in my test in dose of 0.002 gm. per kilogram of rat whereas mercuric chlorid and other soluble salts of mercury in dose of 0.002 gm. per kilogram by intravenous injection are without appreciable effects. The mercurial compounds as a class are far more spirocheticidal than trypanocidal, which subject is discussed in Chapter XXIV.

Owing to the importance of mercurial compounds in the treatment of syphilis, it is not surprising that numerous investigators have attempted to prepare new organic compounds of decreased toxicity for the host with increased toxicity for parasites. It may be stated, however, that no one has as yet succeeded in preparing compounds possessing a notable decrease in toxicity for the body cells along with markedly enhanced trypanocidal and spirocheticidal properties.

Mercurial Compounds in the Treatment of Acute Trypanosomiasis.—The results of trypanocidal tests conducted by Schamberg, Raiziss and myself[1] with *Tr. equiperdum* and various known and new compounds of mercury, all of the latter being prepared in our laboratory, the doses administered being usually as much as the maximum tolerated amounts for white rats by intravenous and intramuscular injection, have yielded generally negative results when compared with the untreated controls included in each experiment. *Tr. equiperdum* was employed routinely, but some compounds were re-tested against *Tr. brucei* and *Tr. lewisi* with similar negative results. Occasionally the development of trypanosomiasis was delayed for one or two days in the treated animals and their lives correspondingly prolonged, but in no instance have we been able to cure infected animals. On numerous occasions I have sought to make the tests more sensitive, that is, more favorable for eliciting trypanocidal properties, by infecting rats with minimal numbers of organisms and administering the mercury compounds at once or within a few hours, but with generally negative results insofar as complete sterilization was concerned. Negative results were likewise observed in the treatment of rats with divided daily doses of mercury compounds; always, however, the infection rapidly gained and proved fatal in almost the same period of time as in untreated controls.

Mercurial Compounds in the Treatment of Chronic Trypanosomiasis. Naturally one would expect better results in the treatment of chronic trypanosomiasis of the rabbit permitting the administration of various mercurials over a long period of time; but in my experience the curative effects have been but slight or doubtful. With the assistance of Miss Anna Rule, I have infected a large series of rabbits with intravenous injections of approximately 500,000,000 *Tr. equiperdum*, beginning treatment about three weeks later. The results summarized in the following table are chosen from the series to illustrate the general findings, and it will be observed that the mercurials administered were without marked or constant curative effect upon the trypanosome infections. Indeed, some of the treated animals succumbed earlier

than the untreated controls, due to a summation of the toxic effects of the mercury and infection:

MERCURIAL COMPOUND IN THE TREATMENT OF CHRONIC TRYPANOSOMIASIS

RABBIT	COMPOUND	ROUTE OF ADMIN.	DOSE PER 60 KILO (GM.)	NUMBER OF DOSES	INTERVAL OF ADMIN.	RESULTS
1	Bichlorid	Intravenous	0.015	6	Daily	Lived 50 days
2	Bichlorid	Intravenous	0.015	3	Daily	Lived 33 days
3	Bichlorid	Intravenous	0.015	4	Twice weekly	Lived 42 days
4	Bichlorid	Intravenous	0.015	6	Twice weekly	Lived 56 days
5	Mercurial ointment	Inunction	0.6	16	Daily	Lived 61 days
6	Mercurial ointment	Inunction	0.6	8	Daily	Lived 51 days
7	Mercurial ointment	Inunction	0.6	7	Daily	Lived 31 days
8	Mercuric salicylate	Intramuscular	0.06	5	Weekly	Lived 63 days
9	Mercuric salicylate	Intramuscular	0.06	4	Weekly	Lived 25 days
10	Control	.....	.....	..	.....	Lived 23 days
11	Control	.....	.....	..	.....	Lived 37 days

All of our animals finally succumbed with the lesions of trypanosomiasis in some of which and notably those of the testicles and prepuce, manp motile trypanosomes were observed by microscopical examination. But it would appear that the mecurials are not entirely without effect as shown by the prolongation of the lives of some receiving the most treatment.

Trypanocidal Activity of Mercurial Compounds in Combined in Vitro-in Vivo Tests.—That mercurial compounds possess trypanocidal activity *in vitro* is readily shown in tests conducted with my combined *in vitro-in vivo* method described in Chapter X. In this method equal parts of the blood of rats heavily infected with *Tr. equiperdum* and varying dilutions of the medicament were mixed and kept in a water bath at 38°C. for 15 minutes when a portion of each mixture was injected into rats to determine the degree of trypanocidal activity. In tests of this kind bichlorid of mercury was sometimes found trypanocidal in final dilutions as high as 1:2500; suspensions of calomel were not trypanocidal in this dilution. A few of the new compounds prepared by Raiziss were trypanocidal in dilutions as high as 1:4000. The superior activity of arsphenamin, however, was shown by the fact that dilutions as high as 1:16,000 to 1:20,000 were trypanocidal in the same tests.

Toxicity and Trypanocidal Activity of Organic Combinations of Mercury and Arsenic.—From the standpoint of the chemotherapy of syphilis it would appear that organic compounds containing both mercury and arsenic would prove of especial interest in view of the possibility of employing both remedies in the one compound. With this in view, Raiziss, Gavron and myself prepared a number of such compounds, the chemistry, toxicity and trypanocidal effects of which are described elsewhere.[2] These new compounds belonged to a class of half complex aromatic organic arsenical compounds into which mercury was introduced. Owing to the presence of 32 to 40 per cent. of mercury the maximum tolerated doses were as low as 5 to 10 milligrams per kilogram of weight. The arsenic content varied from 11 to 15 per cent. in the different compounds and from this standpoint offered encouragement for expecting some trypanocidal activity but in actual tests the group of compounds proved disappointing in this regard due presumably, to the fact that the arsenic was too firmly bound or was not available in an active state.

Influence of Mercurial Compounds upon Antibody Production in Trypanosomiasis.—It is frequently stated that part of the curative activity of mercurial compounds in syphilis is due to the fact they stimulate the body

cells to produce antibodies for *Sp. pallida*. As discussed in Chapter XXIV I have not in my experiments been able to substantiate this theory. I also have considered worth while a study of the possible influence of mercury upon antibody production in trypanosomiasis and especially in relation to the whole question of the influence of drugs upon antibody production in general and more especially in syphilis.

Rabbits were immunized by weekly intravenous injections of a vaccine of *Tr. equiperdum*. Some animals received in addition, the intravenous injection of 0.0005 gm. mercuric chlorid per kilogram of weight four hours after each injection of vaccine. Others received intramuscular injections of salicylate of mercury in olive oil in dose of 0.001 gm. per kilogram (equivalent to 1 grain per 60 kilograms). Five days after each injection, the sera of all rabbits were tested quantitatively for agglutinins, alexo-fixagins and trypanolysins. After nine injections the antibody content of the rabbits receiving vaccine alone was the same as found in the rabbits receiving vaccine and mercurial compounds. In other words, the mercurials given in doses per kilogram of weight corresponding to those ordinarily employed in the treatment of syphilis of human beings, had no influence upon increasing antibodies engendered by immunization with a vaccine of dead trypanosomes.

However, since it appears that antibody production in trypanosomiasis is produced in highest degree by *living* rather than dead trypanosomes, these experiments were not considered conclusive. Rabbits were infected with *Tr. equiperdum;* some were treated with weekly intravenous injections of 0.00005 gm. mercuric chlorid and weekly intramuscular injections of the salicylate in dose of 0.0001 gm. per kilogram of weight. These doses were too small to exert curative influence but were considered sufficient to stimulate antibody production if this were possible. Frequent examination of the serum of each rabbit for agglutinins, alexo-fixagins and trypanolysins during the following eight weeks of the experiment, showed that the untreated controls developed these antibodies to the same degree as the treated animals. I could find no evidence of increased antibody production to be ascribed to the influence of the two compounds employed in this study.

Acquired Resistance of Trypanosomes to Mercury.—Nothing is known regarding the acquisition of resistance to mercurial compounds by trypanosomes. Since the mercurial compounds are much less trypanocidal than arsenical and antimonyl compounds, it would be interesting to determine if trypanosomes naturally resistant to a compound, could have this natural resistance increased *in vitro* and *in vivo*. As stated in Chapter XXIV Akstsu and Noguchi have succeeded in raising the resistance of *Sp. pallida* and other spirochetes to mercuric chlorid *in vitro*, but similar experiments do not appear to have been made with trypanosomes.

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CHAPTER XV

TOXICITY AND TRYPANOCIDAL ACTIVITIES OF COMPOUNDS OF BISMUTH, VANADIUM AND OTHER SUBSTANCES

Since trypanocidal tests have proved so useful in the development of the chemotherapy of syphilis with organic arsenical compounds, it is not surprising that similar tests have been employed in the study of the parasitropic activity of new compounds of other metals for the treatment of this disease. Among attempts of this kind should be mentioned trypanocidal tests with compounds or bismuth, vanadium, thorium and mesothorium.

Toxicity and Trypanocidal Activity of Compounds of Bismuth.—Special interest is to be placed in some of the newer organic compounds of bismuth

and particularly the tartrobismuthates, since these have been found to possess a distinct spirocheticidal properties in various experimental infections and are proving efficacious in the treatment of some cases of syphilis. Here it may be stated, however, that the compounds of bismuth so far produced have proved almost totally devoid of trypanocidal activity. Therefore, these compounds have not proved of any value in the treatment of trypanosomiasis, but they constitute another example of how compounds without trypanocidal activity may be distinctly spirocheticidal. Indeed, as I have repeatedly stated, trypanocidal tests have proved of value only with the organic arsenical compounds for the development of the chemotherapy of syphilis.

Within the last few years, bismuth has commanded considerable attention in the treatment of syphilis and especially sodium and potassium tartrobismuthate. Balzer[1] in 1889, conducted studies on the toxicity of bismuth compounds for dogs and even suggested that they may be useful in the treatment of human syphilis, but it was not until 1916 that Sauton and Robert[2] revived interest in the subject and showed that bismuth compounds possessed curative activity in hen spirillosis.

In 1920 Sazerac and Levaditi[3] observed the curative activity of sodium and potassium tartrobismuthate in rabbit syphilis and also in the natural spirochetic balanitis of this animal (venereal infection due to *Spirocheta cuniculi*), to which I will refer in more detail in Chapter XXV. These investigators also reported that the subcutaneous injection of 0.060 to 0.100 gm. of this double salt per kilogram of weight had a temporary influence upon the course of *Tr. brucei* infections in guinea-pigs.

The trypanosomes generally disappeared from the peripheral blood in 24 to 48 hours after injection, but relapses occurred after 12 to 15 days. Even after the subcutaneous injection of amounts as large as 0.200 gm. per kilo, relapses occurred although these could be prevented for a time by repeating the dose. In one experiment the life of an infected guinea-pig was prolonged in this manner for 80 days, the untreated controls succumbing in about 25 days. Permanent cures, however, were not observed.

Adler[4] observed that the minimal lethal dose of sodium tartrobismuthate was approximately 0.0047 gm. per kilo for mice by subcutaneous injection and 0.062 gm. per kilo for guinea-pigs. When administered to mice infected with *Tr. rhodiensi* in amounts as high as the minimal lethal dose, the trypanosomes disappeared in twenty-four hours with death of the animals in forty-eight hours. Smaller doses had no appreciable effects. In guinea-pigs infected with *Tr. brucei* similar temporary effects were produced with large doses; 0.018 to 0.032 gm. per kilo were followed by relapses in 6 to 12 days with no permanent cures.

In my experiments with the soluble double salt of sodium and potassium tartrobismuthate, the immediate lethal dose for white rats by intravenous injection was found to be approximately 0.180 gm. per kilo. The injection of 0.100 gm. per kilo was usually followed by death in twenty-four hours; 0.050 gm. per kilo was fatal in about forty-eight hours and 0.020 gm. per kilo in seventy-two hours while 0.015 gm. per kilo was about the maximum tolerated dose. The intravenous injection of 0.015 gm. to 0.020 gm. per kilo had no appreciable trypanocidal effects, however, in tests conducted with rats infected with *Tr. equiperdum* according to the technic of my method; similar results were observed with sodium tartrobismuthate, potassium tartrobismuthate and bismuth trioxid.

Similar results have been observed by Myers and Corbitt[5] employing *Tr. equiperdum* in tests conducted after the method of Voegtlin and Smith. The maximum tolerated dose by intravenous injection into white rats for a

period of seven days of four different preparations of soluble preparations of sodium and potassium tartrobismuthate varied from 0.036 to 0.051 gm. per kilogram of weight; the administration of amounts varying from 0.005 to as much as 0.067 gm. per kilo had no appreciable effect upon these experimental infections, except to cause a delay of one to five days in the death of some of the animals with the infection.

In general terms, it may be stated, therefore, that bismuth compounds possess at best but feeble trypanocidal activity; they are, however, decidedly more spirocheticidal and this property will be discussed more fully in Chapter XXV.

Toxicity and Trypanocidal Activity of Compounds of Vanadium.—A thorough study of fourteen of the simple and complex salts of vanadium has been made by Proescher, Seil and Stillians.[6] The toxicity of the majority of these salts was determined by intravenous injection in rabbits and by subcutaneous injection in guinea-pigs, rats and mice. In addition the toxicity of sodium tetravanadate was determined for horses, chickens, pigeons and frogs. The toxicity of the salts studied appeared to depend on their chemical constitution and is influenced by the number of hydroxyl groups attached to a vanadium atom and the presence of the VO_2 group.

Trypanocidal tests employing *Tr. brucei* infections of mice and guinea-pigs showed that the simple vanadium salts such as sodium tetravanadate, anilin vanadate, and vanadyl sulphat may suppress the multiplication of the organisms, or temporarily inhibit the course of the disease. Complete cures could not be effected. The complex vanadium compounds, such as ammonium vanadi-arsenate, effected complete cures in about 16.6 per cent. of animals, while others as ammonium vanado-arsenate and sodium phospho-vanadate were without effect. Best results were observed by a combination of vanadium with arsenical compounds, as arsacetin.

Trypanocidal Activity of Mesothorium, Thorium and Other Substances.—Citron[10] has studied the influence of mesothorium upon pathogenic trypanosomes and found that while exposure to the rays had the effect of reducing their vitality, actual destruction could not be accomplished. I have found the maximum tolerated dose of thorium chlorid for rats to be about 0.050 gm. per kilogram by intravenous injection; doses as large as 0.040 gm. per kilogram, however, were without demonstrable trypanocidal activity in *Tr. equiperdum* infections of white rats. Frouin and Guillaumie[11] have reported that *niobium* is without effect in mice infected with *Tr. brucei;* salts of some of the rate earths as *cerium*, *yttrium* as well as *rhodium chlorid* were found slightly effective by increasing the resistance of animals to infection with trypanosomes.

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CHAPTER XVI

TRYPANOCIDAL PROPERTIES OF DYES, ARSENICAL, MERCURIAL, ANTIMONYL AND BISMUTH COMPOUNDS IN VITRO

In Chapter X were described methods for determining the trypanocidal activity of substances *in vitro*. Determinations of this kind are sometimes of value, not only in relation to studies bearing upon the mechanism of trypanocidal activity of different substances, but also for furnishing data in connection with systematic studies in chemotherapy or the relationship between chemical constitution and biological activity.

Not all substances possessing high trypanocidal activity *in vitro* are trypanocidal *in vivo;* notable examples are the group of mercurial compounds which, owing to their high toxicity for the host, cannot be given animals in sufficiently large amounts to more than merely influence the course of trypanosomiasis for a day or two. On the other hand, substances possessing very little or no trypanocidal activity *in vitro* may be more active *in vivo;* in this connection it is interesting to recall that Ehrlich and Shiga made the mistake in 1904 of discarding atoxyl because their work was confined to the test-tube with negative results.

Trypanocidal tests *in vitro* may be confined to a study of the influence of the medicament upon the motility of trypanosomes or include tests for actual and complete destruction by injecting portions of the mixtures into the peritoneal cavities of mice and rats and subsequently studying the animals for blood infections over a period of not less than two weeks. Methods of this kind are required because the parasites cannot be successfully cultivated in artificial media with sufficient regularity to yield acceptable data of trypanocidal activity.

Trypanocidal tests *in vitro* have not been conducted as frequently as *in vitro* spirocheticidal tests, especially tests employing *Sp. obermeieri* and *Sp. gallinarum.* Browning[1] tested a number of the dyes *in vitro* against *Tr. brucei* and found the benzidin derivatives as trypan-red, trypan-blue and trypan-violet practically inactive, whereas the basic triphenylmethane dyes, such as parafuchsin, ethyl-violet and Dobner's violet were found actively trypanocidal. Atoxyl in 1:100 concentration was only slightly effective against motility, while arsacetin in concentration equal to 1 per cent. of the free acid was without any effect after an exposure of two hours at room temperature.

In Table 3 are summarized the results of *in vitro* tests conducted with a number of dyes and different compounds of arsenic, mercury, antimony and bismuth. These tests were conducted by mixing in small test-tubes 0.5 c.c. of citrated rat blood containing large numbers of actively motile *Tr. equiperdum* with 0.5 c.c. of varying dilutions of the compound under study. These mixtures with the controls were placed in a water bath at 37°C. for 15 minutes and the motility of the trypanosomes examined at the end of 5, 10 and 15 minutes. At the end of 15 minutes 0.5 c.c. of the controls and dilutions of compounds showing no motile trypanosomes were injected into rats intraperitoneally and the animals observed for two weeks. The + sign in the table indicates that trypanosomiasis developed as detected by daily examinations of tail blood.

Methylene blue, atoxyl, sodium arsenate, tryparsamid, and sodium cacodylate did not produce trypanolysis in final dilutions of 1:200, but all of the other compounds shown in the table dissolved the trypanosomes in solutions just a little stronger than those producing loss of motility. In the saline controls the trypanosomes were well preserved and motile at the end of fifteen minutes and proved highly infective for rats.

TABLE 3

COMPOUND	MOTILITY 15 MIN.		RAT INOCULATION TESTS	
	PRESENT	ABSENT	DILUTION	RESULTS
Trypan-red	1:20,000	1:10,000	1:10,000	+
Trypan-blue	0	1:20,000	1:10,000	+
Methylene-blue	0	1:500	1:500	−
Methyl-violet	1:60,000	1:40,000	1:40,000	−
Fuchsin (acid)	1:10,000	1:5000	1:5000	−
Safranin	0	1:80,000	1:40,000	−
Arsenious acid	1:10,000	1:20,000	1:5000	−
Sodium arsenite	1:80,000	1:40,000	1:20,000	−
Sodium arsenate	0	1:200	1:200	−
Atoxyl	0	1:200	1:200	−
Sodium cacodylate	0	1:200	1:100	+
Tryparsamid	0	1:200	1:200	−
Arsphenamin	1:320,000	1:160,000	1:160,000	−
Neoarsphenamin	1:320,000	1:160,000	1:160,000	−
Silver arsphenamin	0	1:320,000	1:320,000	−
Mercuric chlorid	0	1:5,000,000	1:2,500,000	+
Tartar emetic	0	1:10,000,000	1:5,000,000	+
Sodium and potassium tartro-bismuthate	1:200	1:100	1:100	−
Saline controls	+	0	−	+

As shown in the table tartar emetic proved most strongly trypanocidal *in vitro*, followed by mercury, arsphenamin and neoarsphenamin. The pentavalent organic and inorganic compounds of arsenic were very much less active; bismuth and the dyes were likewise relatively much less strongly trypanocidal.

In the majority of instances dilutions sufficient for destroying motility were likewise trypanocidal as determined by the rat test; exceptions occurred with trypan-red trypan-blue, sodium cacodylate, mercuric chlorid and tartar emetic. Loss of motility is not always an indication of death of the organisms, but usually this is the case; apparently some compounds may destroy motility in dilutions much higher than necessary for trypanocidal activity, but of course, the chances of experimental error are considerable inasmuch as motile parasites may escape detection in the microscopical examination of mixtures.

In a broad and general manner there is some relationship between activity *in vitro* and *in vivo* although some exceptions stand out rather sharply. For example, the trypanocidal properties of the dyes, arsenious acid, atoxyl, sodium arsenate, sodium arsenite, sodium cacodylate, tryparsamid, sodium and potassium tartro-bismuthate, are rather low *in vivo* and their influence upon motility *in vitro* is likewise relatively slight. On the other hand the activity of the trivalent organic arsenical compounds, as arsphenamin, neoarsphenamin and silver arsphenamin, is very high *in vivo* and likewise *in vitro*. Mercury, however, has relatively low trypanocidal activity *in vivo*, due in part to high toxicity preventing the administration of large doses, but

in vitro mercuric chlorid possesses in high degree the power of destroying motility, although this is not always a sign of trypanocidal activity. In other words, *compounds possessing low trypanotoxic properties in vitro are likely to have low trypanocidal activity in vivo;* however, compounds may be highly trypanotoxic *in vitro* and yet relatively inert *in vivo* when administered in the maximum tolerated amounts.

Chronic Infections with Trypanosomes Which Have Been Treated in Vitro.— Of considerable interest and significance from the standpoint of the possible development of an efficient method for active immunization in trypanosomiasis, are observations showing that the infections produced by trypanosomes previously exposed to various drugs *in vitro*, tend to become prolonged and chronic. These results are due either to a reduction of the virulence of the organisms *in vitro*, which is transmitted to new generations *in vivo*, or to the fact that the animal host is unable to engender sufficient immunity to hold the infection in check and neutralize the trypanotoxins for relatively long periods of time.

Thus Browning has observed that *Tr. brucei* in defibrinated mouse blood, exposed for tweny minutes to dilutions of parafuchsin in sugar-salt solution varying from 1:9000 to 1:300,000 and then injected into mice, required more days to appear in the peripheral blood than the controls of trypanosomes in the sugar-saline solution alone; in one instance a dilution of parafuchsin 1:300,000 inhibited the development of trypanosomiasis for three days, showing how delicate this biological test is as a means of detecting damage of the parasites. Trypanosomes exposed to parafuchsin of 1:9000 to 1:80,000 required in some instance as long as 15 days to produce infections in mice and in one instance, the infection persisted for 114 days, as compared with the usual period of life of about 4 to 5 days when mice were infected with the controls. In my *in vitro* experiments it was found that *Tr. equiperdum,* exposed to solutions of trypan-red, trypan-blue, sodium cacodylate, mercuric chlorid and tartar emetic sufficient to destroy motility were sometimes infective, but the invasiveness and virulence of these trypanosomes were appreciably reduced.

This condition of semi-immunity or reduction in virulence is analogous to what Koch produced by inoculating cattle with attenuated trypanosomes. In my opinion it is primarily due to sufficient injury *in vitro* to impair the rapidity of proliferation of the parasites *in vivo;* apparently the first parasiticidal effect to occur is this impairment of the powers of proliferation or propogation *in vivo.* Under these circumstances the animal host is better able to engender a sufficient degree of active immunity to hold the infection in check and neutralize trypanotoxins; as I will discuss in a later chapter, there is evidence to show that immunity in trypanosomiasis is engendered only by living organisms as is true of the immunity in syphilis engendered only by living spirochetes.

Under the conditions of reduced proliferative powers and consequent reduction of evasiveness on the part of the parasites, the host is enabled to engender some immunity principles and, as previously stated, this may have a bearing upon the development of a successful plan for vaccination against trypanosome diseases.

REFERENCE

1. Jour. Path. and Bacteriol., 1908, 12, 166.

CHAPTER XVII

IMMUNITY IN TRYPANOSOMIASIS OF MAN AND THE LOWER ANIMALS IN RELATION TO CHEMOTHERAPY

Comparatively little has been done in the study of natural and acquired immunity in trypanosomiasis of human beings and the lower animals, as compared with immunological studies in syphilis and bacterial infections. Probably in large part this is due to the fact that the importance of the subject has not been sufficiently great, because trypanosomiasis of human beings is fortunately confined to portions of Africa and to Chagas disease of Brazil instead of being world wide in distribution.

Natural Immunity in Trypanosomiasis.—Apparently human beings possess a high degree of natural immunity to practically all of the trypanosome diseases of the lower animals, excepting those produced by *Tr. gambiensi* and *Tr. rhodesiense.* Whether this is due in part to the fact that the flies, fleas and bugs, acting as intermediate hosts for these pathogenic trypanosomes of the lower animals, do not feed on or bite human beings, cannot be definitely stated; it is more probable that the blood and fixed tissues of human beings possess natural resistance or immunity. The nature of this natural immunity, however, is not understood, just as the mechanism of natural immunity of man to many of the bacterial diseases of the lower animals and of the lower animals to syphilis and many of the bacterial diseases of human beings, is not understood. The few investigations that have been made indicate that the trypanosomes are unable to survive and proliferate in the blood of the immune species, either as the result of the presence and activity of trypanolysins or the lack of suitable vitamins and pabulum (athreptic immunity).

Presumably all human beings are susceptible to infection with virulent strains of *Tr. gambiensi* and *Tr. rhodesiense;* in certain districts of Africa, however, notably in Nigeria, where sleeping sickness has been long endemic, the disease is less virulent and much more amenable to treatment than in Uganda, on the Congo and in Angola. This suggests that in time the population may either develop some degree of immunity or, rather, that the virulence of the trypanosomes is reduced, analogous to the gradual reduction in the virulence of syphilis during the past four hundred years in those peoples among whom the disease has been endemic. In some parts of Africa whole native villages have been depopulated by trypanosomiasis. Europeans are likewise susceptible and especially so are the younger individuals of both races. Instances of presumably individual immunity are not lacking, but these are probably due to protection against the bites of infected flies rather than an inherent and efficient degree of natural immunity. Even the offspring of recovered cases of African trypanosomiasis are susceptible to infection.

Acquired Immunity in Trypanosomiasis.—The question naturally arises whether infected human beings ever recover spontaneously from trypanosomiasis. Manson and others state that, although spontaneous recovery may take place in the early stages of trypanosomiasis, when the disease has arrived at the late stage death is inevitable. The same is true of most of the trypanosome diseases, natural and experimental, of the lower animals. In

other words human beings and the lower animals are not able to engender sufficient immunity principles in the blood, lymph and fixed tissues to effectually bring about spontaneous recovery, in the majority of instances, in virulent trypanosome infections. This is in conformity with our experience and information in immunity in protozoan infections in general. For example, in malaria and syphilis, sufficient immunological factors may be engendered and mobilized to throw the infectious agents into periods of latency and reduced virulence for varying periods of time, but without bringing about complete recovery except, possibly, in doubtful and exceptional instances.

Antibody Production in Trypanosomiasis.—In trypanosomiasis of human beings and the lower animals antibodies may be produced in the blood but seldom to a high degree. Agglutinins may be found and especially in the terminal stages; likewise complement-fixing antibodies (alexo-fixagins) and trypanolysins. The alexo-fixagins are especially apt to be found in the blood-serum and notably in dourine of horses, but it may be that these are not true antibodies in the sense of being destructive for the parasites; rather they may be analogous to the "reagin" produced in syphilis and responsible for the complement-fixation and flocculation reactions of this disease. In my opinion this "reagin" in syphilis may be regarded as a reactionary product of the lymphocytes and other cells surrounding *Sp. pallida*, without possessing more than a feeble destructive effect upon the spirochetes; and since spontaneous recovery in trypanosomiasis of human beings and the lower animals is exceptional, despite the presence of these antibodies, it is possible that the alexo-fixagins of trypanosomiasis have a similar origin and significance.

In acute trypanosomiasis of mice and rats fatal in 3 to 10 days, antibodies are not usually found at all owing to the acuteness and early fatal character of the infection. If, however, the infection is held in check by the administration of arsphenamin or neoarsphenamin in such doses as produce relapses without complete cure, the animals can be kept alive for weeks, and under these circumstances complement-fixing antibodies gradually appear being first demonstrable in my experiments about seven days after infection. In rabbits and guinea-pigs the production of complement-fixing antibodies also takes place, first appearing in *Tr. equiperdum* infections of rabbits about five days after infection.

Rôle of Immunity in the Chemotherapy of Acute and Chronic Trypanosomiasis.—It will be understood, therefore, that in acute trypanosomiasis of the mouse and rat there is practically no chance for the production of immunity principles, since the production even in chronic infections tends to be slight and tardy. There is therefore, no reason for believing that immunity principles aid in the cure of acute trypanosomiasis, when single large doses of arsenical, antimonyl, bismuth or other trypanocidal compounds are given; there is not sufficient time for the intervention of immunological processes, inasmuch as sterilization is a matter of hours instead of days and is to be attributed to the direct trypanocidal activity of reduction or oxidation products of the chemical compounds.

In chronic trypanosomiasis, however, immunity undoubtedly plays a part in holding the infection in check and may even produce varying periods of latency. This is especially true of infections treated with substerilizing doses of the dyes, arsenicals and other trypanocidal compounds. Under these conditions the medicaments appear to aid in the production of antigenic substances from living trypanosomes; I do not believe that the drugs actually stimulate the antibody-producing tissues to any appreciable degree although in large doses they may depress them, but may aid in the production of antibodies by releasing antigenic substances from the parasites.

Immunological processes may likewise play some part in the temporary period of latency in trypanosomiasis following the administration of subcurative doses of various trypanocidal agents which is discussed in a succeeding section.

Immunity Following Cure in Trypanosomiasis.—While some degree of immunity may develop in chronic trypanosomiasis of both human beings and the lower animals, which may be accompanied by the production of antibodies in the serum and spinal fluid, this immunity is usually not sufficient for bringing about complete and spontaneous cure in virulent infections. Rats infected with *Tr. lewisi*, however, generally recover spontaneously after 4 to 8 weeks and these are usually immune to re-infection with *Tr. lewisi*, although just as susceptible as normal control rats to such pathogenic parasites as *Tr. brucei* and *Tr. equiperdum.* This immunity, however, is not absolute. In my experience it tends to diminish after 18 days and the animals may sometimes be infected at this time by the intraperitoneal injection of very large numbers of the same strain of *Tr. lewisi.*

A very curious phenomenon is the temporary immunity following the cure of trypanosomiasis in mice and rats. In 1904, Ehrlich and Shiga[1] discovered that mice infected with *Tr. equinum* and cured with trypan-red, were resistant to re-infection with these trypanosomes for about 20 days. Experiments showed that the immunity was not due to unexcreted dye, as mice treated with dye alone in the same way were susceptible. Furthermore, the immunity after cure was specific only for the trypanosomes employed; the mice were just as susceptible as normal controls to other strains.

Terry[2] made a particularly thorough study of this immunity and confirmed these observations in experiments employing mice infected with four different strains of pathogenic trypanosomes and a number of dye and arsenical compounds and combinations of these.

If the curative activity of single large doses of trypanocidal agents, including the dyes and compounds of arsenic, antimony, etc., are due to the production of new reduction- or oxidation-products *in vivo* having a direct trypanotoxic activity without the aid or intervention of antibodies, what is the nature of this temporary period of resistance to re-infection after a cure has been brought about? Furthermore, what is the nature of the mechanism whereby, after the administration of a subcurative dose, trypanosomes disappear for a time and then reappear as a recurrent or relapsing infection with a fatal termination? Are these effects due to the presence and temporary activity of unexcreted medicament? Are they due to immunity principles? Or are they due to a combination of these?

Influence of Unexcreted Medicament upon Temporary Resistance.—If they were due to unexcreted medicament it should be possible to give the drugs to normal rats and then find them resistant to infection with trypanosomes for a varying period thereafter. Ehrlich found that this was not the case with dyes and Terry confirmed his results; that is, dyes injected into normal mice did not protect them against infection, whereas infected mice cured with the same doses remained refractory to re-infection for a brief time thereafter.

Are similar results to be observed in the case of the organic arsenical compounds? Terry found that atoxyl and arsacetin acted in much the same way, but the results were not as conclusive as observed with the dyes or with combinations of the dyes and arsenical compounds.

I have experimented with arsphenamin and neoarsphenamin with the following results. When arsphenamin was administered to rats by intra-

venous infection in dose of 0.004 to 0.010 gm. per kilogram 24 *hours before infection*, trypanosomiasis usually developed as in the untreated controls; whereas, when administered just before infection or 24 hours after, infection did not occur. In other words, these amounts were apparently excreted within 24 hours and similar results were observed with 0.005 to 0.015 gm. of neoarsphenamin per kilogram. However, the injection of 0.020 gm. of arsphenamin or 0.040 gm. of neoarsphenamin per kilogram 24 hours before infection generally prevented trypanosomiasis, indicating that a sufficient amount remained in the rats at the end of 24 hours to kill the trypanosomes injected intraperitoneally.

Additional experiments consisting of injecting the drugs 48 and 72 hours before infection yielded practically the same results; that is, unless the amounts administered were very large, the animals could be infected almost as readily as untreated controls. These experiments demonstrated, therefore, that the period during which ordinary doses of arsphenamin and neoarsphenamin remains curative after intravenous injection is quite brief and if a temporary resistance or immunity to re-infection follows a cure it cannot be ascribed to the activity of unexcreted medicament. Furthermore, the period of two to fifteen days following the injection of 0.001 to 0.003 gm. of these compounds per kilogram, during which trypanosomes are not to be found in the blood, can hardly be ascribed to the influence of unexcreted drug.

In this connection I may state that in additional experiments I injected rabbits every three to four days with 0.020 gm. of arsphenamin per kilogram for six doses in order to store some of the compound in the tissues. The blood and heavy emulsions of the brain, liver, kidneys, spleen and muscles of each animal were then injected in large doses into infected rats, to determine if they contained enough of the arsphenamin or its products to influence the infections. With the exception of the liver preparations the results were negative. In all instances the liver suspensions retarded the development of trypanosomes to a slightly greater degree than the controls of liver from untreated animals, but the differences were not marked and indicated at best but a slightly increased trypanocidal activity of the liver extracts of the arsphenaminized animals. In other words, the results showed that the stored arsenic is not therapeutically active, or if so, but in a slight degree.

Resistance to Re-infection Following the Cure of Trypanosomiasis by Arsphenamin and Neoarsphenamin.—I have not observed in trypanosomiasis of rats the same degree of so-called immunity following cure with arsphenamin and neoarsphenamin that Terry observed in mice cured with trypan-red, atoxyl and mixtures of these, or in mice infected with *Tr. brucei* and cured with atoxyl and arsacetin reported by Browning.[3]

Arsphenamin in dose of 0.020 gm. per kilogram and neoarsphenamin in dose of 0.040 gm. intravenously 24 hours before the intraperitoneal injection of 500,000 *Tr. equiperdum* generally prevented infection. When these compounds were injected three or more days before inoculation, infection occurred as in the untreated controls.

When rats were first infected and treated with the same doses about 40 hours later when trypanosomes were in the blood, the organisms disappeared in the following 24 hours. If these animals were now re-inoculated with trypanosomes at the end of this first day, infection did not occur; but if they were re-inoculated three or more days after treatment, infection always occurred, although in some instances the time of first appearance of the trypanosomes in the peripheral blood was delayed beyond the controls. This showed that there was practically none or but a slight degree of immu-

nity following the cure of 40-hour *Tr. equiperdum* infections of rats with arsphenamin and neoarsphenamin, since the results could be ascribed in large part to unexcreted drugs.

When rats were first infected and treated 72 hours later at a time when the blood was swarming with trypanosomes, it was found that the organisms disappeared in the following 24 hours. With arsphenamin-treated rats re-infection could not ordinarily be produced by inoculating trypanosomes within a period of four days following the treatment, but with neoarsphenamin re-infection could be accomplished in three days after treatment. This showed that the cure of heavy trypanosome infections with arsphenamin was followed by a brief period of resistance to re-infection; with neoarsphenamin this period was shorter and less definite.

Nature of the Temporary Immunity Following Cure in Trypanosomiais.—What is the nature of this temporary resistance? Ehrlich, Terry and others have called it an immunity and some investigators claim that the blood-serum during this period is trypanocidal *in vitro* and slightly curative *in vivo*.

This immunity, however, is of a very curious nature and absolutely specific for the particular strain used in producing the infection. For example, Browning infected mice with a parafuchsin-resistant strain of *Tr. brucei* and after curing the animals with atoxyl found that they could not be re-infected with the same parafuchsin-resistant strain for about ten days, but that they could be readily infected with an atoxyl-resistant strain of *Tr. brucei*. One would expect that an immunity following the cure of *Tr. brucei* infection would be effective against all strains of this trypanosome, but this is not the case; similar results were observed by Terry with different strains of *Tr. equinum*.

Apparently the trypanocidal drugs liberate from trypanosomes *in vivo* antigenic substances capable of engendering temporary periods of highly specific immunity. Arsphenamin and neoarsphenamin, however, are apparently less active in this particular than atoxyl, arsacetin and arsenophenylglycin.

As will be discussed presently, with dead trypanosomes practically no immunity results after the administration of drugs; *apparently, living trypanosomes are required for the production of the antigenic principles,* but even with living *Tr. equiperdum* infections of rats, arsphenamin and neoarsphenamin do not produce more than the briefest period of immunity after cure.

Relation of Chemotherapy to the Production of Immunity by Living Trypanosomes.—As just stated, the administration of arsphenamin and neoarsphenamin do not appear to aid appreciably in the production of antibodies by dead trypanosomes. But do drugs, and particularly the arsenical compounds, increase antibody-production by living trypanosomes? This is the question of paramount importance, because immunity in trypanosomiasis is very similar to immunity in syphilis in that the presence of the *living* parasites in the tissues appears to be required for the production of states of resistance to re-infection and immunity.

In my experiments, rabbits infected with *Tr. equiperdum* and treated weekly with intravenous injections of 0.0005 gm. arsphenamin and neoarsphenamin per kilogram (about one tenth of the minimal curative dose) have actually shown the production of more agglutinins, alexo-fixagins and trypanolysins than untreated controls, although the amounts of these antibodies were small and their production was tardy, being but slightly more than recorded above as engendered by vaccines of dead trypanosomes.

An additional experiment was conducted by infecting 8 rats with *Tr. equiperdum.* Two days later these showed approximately 750,000,000 per cubic millimeter of blood. Four of these rats were treated with the intravenous injection of 0.020 gm. of neoarsphenamin per kilogram, sufficient to effect sterilization. At daily intervals thereafter one rat was re-inoculated and on the third day after treatment it was found possible to re-infect, the immunity having lasted but three days after cure.

The four remaining rats were killed when the blood contained about 420,000,000 trypanosomes per cubic millimeter and the blood of each collected into citrate solution and the trypanosomes killed by heating in a water bath at 60°C. for one hour. Each blood-vaccine was now injected into a fresh rat and these were then treated with the same dose of neoarsphenamin to determine if an immunity would follow and if so, how it compared with that produced with the destruction of living organisms. A rat was re-inoculated, therefore, at daily intervals with living trypanosomes and infection occurred each time. At the same time 4 more normal rats were injected with the drug alone in the same dose and a rat inoculated each day to determine the influence of unexcreted drug. In this series it was possible to infect on the first and each of the three succeeding days after treatment. The results, therefore, may be summarized as follows:

(*a*) Resistance to infection following the injection of drug alone was less than one day.

(*b*) Duration of resistance to infection following treatment of living trypanosomes was about ten days.

(*c*) Duration of resistance to infection following treatment of dead trypanosomes was less than twenty-four hours.

These experiments showed, therefore, that a temporary immunity follows the cure of rats infected with living *Tr. equiperdum* by the intravenous injection of arsphenamin and neoarsphenamin. *They have also shown what has heretofore not been demonstrated, namely, that the immunity does not follow if the rats are first inoculated with dead instead of living trypanosomes before treatment is given. In other words, the development of the immunity requires that living trypanosomes shall be destroyed in the animal by some trypanocidal agent.*

Whether or not immunity follows the cure of human beings infected with *Tr. gambiensi* I am unable to state. Presumably it does for a temporary period at least, if the results of experiments in the lower animals are applicable to human trypanosomiasis. I have not been able to find records of cases of re-infection of human beings where the question of relapse could be definitely excluded. From the standpoint of animal experimentation, I believe it may be reliably state that one attack of trypanosomiasis followed by cure, does not confer an immunity against subsequent attacks with the same or other species of trypanosomes.

Duration of Infection in Relation to the Duration of the Immunity Following Cure.—Additional experiments have shown that to a slight degree the longer the trypanosomes are allowed to live in the rats before cure is effected, the longer and more definite is the resulting immunity. However, there is no definite relationship between the degree and duration of the infection and the degree and duration of the immunity following cure. Terry has observed the immunity in mice following the infection of trypanocidal agents when the infection was so light that trypanosomes could not be found in the peripheral blood.

Mechanism of Relapsing Infections.—As previously mentioned rats infected with *Tr. equiperdum* and treated with a curative dose of either arsphenamin or neoarsphenamin can be re-infected with these trypanosomes two or three days later. However, if infected rats are treated with one half of the curative doses of these compounds the trypanosomes disappear and stay out of the peripheral blood for many days, even as long as fifteen days. In other words the animals are protected against the trypanosomes of the first infection for a week or more but are susceptible to re-inoculation with fresh trypanosomes within a day or two.

This period of temporary cure is probably due in largest part to the fact that the proliferative power of the trypanosomes was injured by the drugs, so that the organisms are unable to multiply as rapidly as usual; in part it is also probably due to the immunity described above.

However, it cannot be due entirely to immunity, because, if so, the animals should be refractory to re-inoculation with small numbers of the

same trypanosomes for about as long as the period of temporary cure. Furthermore, when the trypanosomes appear again in the relapsing or recurring infection, they usually kill the mouse or rat just as rapidly as freshly inoculated trypanosomes unless treatment is given; in some instances, however, the infection persists much longer, as if the trypanosomes were held in check by immunity principles, their rate of multiplication reduced, or as if the animal had developed a tolerance for their toxic products.

As a general rule the duration of temporary cure bears a relation to the size of the dose of trypanocidal drug; this suggests that unexcreted drug may likewise exert an influence upon the duration of this period. If this is true, *the trypanosomes are apparently more susceptible to the unexcreted medicament than are freshly inoculated organisms of the same strain.*

The duration of the period of temporary cure, before relapses occur, can be greatly prolonged by repeated injections of trypanocidal agents. These effects are probably due to injury of proliferating mechanism of the trypanosomes, to the production of immunity and increase of tolerance of the body cells to trypanotoxins. Finally, however, the organisms may develop a "resistance" or "fastness" to the compound and produce fatal relapses despite treatment unless an unrelated drug is employed.

Of considerable interst in this connection is the condition where the trypanosomes may be found during the temporary periods of latency in untreated infections of guinea-pigs and rabbits and more especially in rats and mice treated with substerilizing doses of trypanocidal agents, when organisms are not to be found in the blood. It is generally stated that trypanosomes are to be found in the spleen and bone marrow under these conditions.

In my experiments rats were treated with subcurative doses of arsphenamin, neoarsphenamin and tartar emetic about two days after infection and at a time when the blood contained large numbers. For three or more days following none were to be found in the blood and during this period animals were killed and emulsions of the brain, spleen, liver and bone marrow prepared. These were injected into normal rats intraperitoneally and the animals examined daily for 17 days. The results showed that during latency trypanosomes may be present in the blood and internal organs, especially the liver, spleen and bone marrow.

Vaccines in the Prophylaxis and Treatment of Trypanosomiasis.—In connection with this subject of immunity in trypanosomiasis, it may not be amiss to briefly discuss the subject of active immunization. To the best of my knowledge vaccines have not been employed for prophylactic or curative purposes on a large scale, although they are not difficult to prepare and would possess great value in the prevention of trypanosomiasis of human beings and the lower animals if it could be shown that their administration was followed by the production of antibodies and immunity.

Judged by the clinical course of trypanosomiasis, little or nothing is to be expected, however, from vaccine prophylaxis and treatment. In trypanosomiasis of human beings, as well as in experimental infections of guinea pigs and rabbits, the progress of the disease is sufficiently tardy to permit the development of curative immunity if the body cells were able to bring this about; since, however, the living trypanosomes are unable to engender this immunity, the chances are slight that a vaccine of dead parasites would be more antigenic.

I have immunized rabbits over a period of three to four months with weekly doses of a vaccine of *Tr. equiperdum* by intravenous injection and studied the blood of each animal after each injection for agglutinins, alexo-fixagins and trypanolysins. These doses ranged

from approximately 1,000,000 to 5,000,000 dead trypanosomes per animal (average weight 1500 grams). At the end of the period of immunization each animal was infected with *Tr. equiperdum* and the development of trypanosomiasis compared with infected controls. The sera of these immunized animals was also employed in the treatment of rats infected with *Tr. equiperdum*. The results are summarized as follows: (1) Vaccines engender but slight amounts of agglutinins and complement-fixing antibodies; (2) Vaccines produce but small amounts of trypanolysins; (3) Rabbit immune sera possess but slightly greater protective and curative properties than normal rabbit sera and (4) Immunized rabbits were but slightly more resistant to infection with *Tr. equiperdum* than normal rabbits.

These experiments indicate, therefore, that vaccines of *Tr. equiperdum* engender but slight degrees of immunity. It is possible that living trypanosomes would prove more antigenic, but owing to the extreme susceptibility of animals to virulent strains, immunization of this kind would prove dangerous. Terry has recorded the rather high and very specific nature of the immune sera of goats infected with strains of *Tr. evansi* and serum has been employed by numerous investigators in the treatment of experimental trypanosomiasis of the lower animals. The fundamental reason for failure of vaccine prophylaxis of trypanosomiasis is probably that in this, as in other protozoan infections, immunity and resistance develop slowly and tardily, if at all, in immunization with *dead* parasites. In syphilis vaccines of dead spirochetes are likewise but feebly antigenic; immunity in syphilis requires in the tissues the presence of living spirochetes, although they may be so few and latent that symptoms are not present. Almost the same phenomenon is seen in tuberculosis; apparently only living tubercle bacilli are capable of exciting allergic sensitization of the tissues and vaccines of non-virulent but living bacilli yield best results in immunization. Apparently the same is true of trypanosomiasis; only the living parasites appear capable of producing sufficient antigenic stimulation to engender an immunity and this process is greatly aided and hastened by the administration of a trypanocidal substance, as if the antigenic substance is derived from the lysis of the bodies of the trypanosomes, this process being aided by bringing about their death in the living animal followed by dissolution of the dead parasites by means of autolytic or serum-enzymes. With human beings as with the lower animals, recovery from infection with virulent trypanosomes is not to be expected without medicinal aid, and for this reason the development of a successful method of medicinal treatment is imperative.

Influence of Drugs upon Antibody Production by Vaccines in Trypanosomiasis.—One theory of the mechanism of the curative action of arsphenamin, neoarsphenamin and mercury in syphilis is that these compounds stimulate the body-cells to produce antibodies which are destructive for the spirochetes rather than the reduction-products of the compounds themselves. Naturally this theory is given considerable weight by the immunity following the cure of trypanosomiasis discussed above. As mentioned in the preceding chapter on the mechanism of the trypanocidal activity of organic arsenical and antimony compounds in trypanosomiasis, there is no evidence to show that the death of the trypanosomes is brought about by antibodies rather than by the reduction-products of the compounds themselves when a single curative dose of the drug is given. Under these conditions trypanocidal activity is due to the drugs—if not directly by the drugs as injected, then at least by new compounds produced by their reduction or oxidation in the tissues—because cure is brought about altogether too quickly to afford time for the production of immune substances. However, as will be shortly discussed, when trypanocidal drugs are given in divided doses, there is reason for believing that the resulting cure is aided by immune processes.

In connection with the experiments summarized above on the production of immunity in rabbits by injections of a vaccine of *Tr. equiperdum*, exactly similar tests were conducted at the same time in which the rabbits were injected with the same vaccine in the same way and at the same time received intravenous injections of 0.010 gm. arsphenamin and 0.015 gm. neoarsphenamin per kilo of weight to determine if these compounds would aid and increase the production of antibodies and immunity. Exactly the same tests for agglutinins, alexo-fixagins and trypanolysins were conducted; after twelve injections of vaccine and drug the results were identical with those summarized above in the case of rabbits receiving only the vaccine. The animals were also finally infected with living trypanosomes as described above, and the appearance of trypanosomes in the blood and the appearance of symptoms and lesions of trypanosomiasis occurred in practically the same time and manner as among the rabbits receiving only the vaccine. These experiments showed that the drugs (administered in the usual therapeutic doses employed for the treatment of syphilis) were without the slightest effect in aiding the production of demonstrable antibodies in the serum when administered along with a dead vaccine.

In other words *the administration of these drugs does not aid in the production of immune substances and immunity when administered along with dead vaccines; they do, however, aid in the production of immunity and cure when administered during the course of trypanomiasis*, as shown by the immunity in relapses and following cure. For this reason it is highly probable that in the treatment of trypanosomiasis the cure that follows a single large dose of trypanocidal agent is brought about by the drug itself; but in cures brought about by the administration of divided doses, cure is aided by the production of immunity principles.

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CHAPTER XVIII

COMPARATIVE STUDY OF METHODS FOR THE ADMINISTRATION OF TRYPANOCIDAL COMPOUNDS IN RELATION TO THE CHEMOTHERAPY OF TRYPANOSOMIASIS

In the preceding chapters bearing upon the chemotherapy of experimental trypanosomiasis, the relative values of the different drugs were largely expressed upon the basis of single maximum tolerated and single minimal curative doses per kilogram of body weight. In the majority of instances the compounds were administered by intravenous injection in order to secure as decisive results as possible, and acute infections of mice and rats were employed to a greater extent than chronic infections of rabbits. The results are of great value not only in the interests of the advancement of the pure science of chemotherapy, but also because the data obtained is applicable in a broad and general manner to the treatment of trypanosome diseases of human beings and the lower animals. It is true, as will be shortly discussed, that in the treatment of chronic trypanosomiasis the questions of penetration

of the medicament into the lesions, as well as the modifying influences of rate or excretion, debilitated condition of the host and pathological changes in the various tissues and organs, require due consideration, but in a general way those medicaments yielding the best chemotherapeutic indices in acute trypanosomiasis are also most likely to yield best results in chronic infections and particularly if they may be given by intramuscular and subcutaneous injection as well as intravenously.

Limited Value of Therapie Magna Sterilisans in Trypanosome Diseases. While it is true that single large doses of various trypanocidal compounds are curative for the acute and even chronic experimental trypanosome infections of the mouse, rat and rabbit, yet this method has a very limited application in the treatment of African trypanosomiasis of human beings and natural iinfections of the lower animals. It is only exceptionally in human beings that treatment is undertaken sufficiently early after infection to hold out hope for complete cure with a single dose of drug; in the great majority of instances the lymphatic glands and other tissues are already involved, even though the spinal fluid shows no changes of increased cells and proteins. Single large doses may clear the blood stream, but relapses are likely to occur. Even under the most favorable conditions when treatment is possible soon after infection, the majority of physicians would hesitate to administer the doses required, as indicated by the results of animal experiments. For example, the single minimal curative dose of tryparsanid for rabbits within 5 to 10 days after infection with *Tr. gambiensi* would be about 0.2 gm. per kilogram; this is equivalent to 12 grams for a 60 kilogram adult (about 130 pounds), but one would naturally hesitate to administer a single dose of this size either by intravenous or intramuscular injection, even though the subject were in good general condition. A much better plan would be the administration of smaller amounts at intervals sufficiently close that an injection is given before the curative effects of the former treatment have ceased. *Therapie magna sterilisans* is possible under experimental conditions but rarely in natural trypanosome diseases.

Application of the Results of the Chemotherapy of Experimental Trypanosomiasis to the Treatment of Trypanosome Diseases of Man and the Lower Animals.—The results observed in the chemotherapy of experimental trypanosomiasis have, however, a direct application in the treatment of trypanosome diseases of human beings and the lower animals to the extent that compounds possessing the highest chemotherapeutic indices in experimental infections are most likely to be efficacious and to be preferred.

Unfortunately, however, data regarding dosage in the chemotherapy of experimental trypanosomiasis is not directly applicable to human beings or even the lower animals with trypanosome diseases. While body weight is a much better guide than age for regulating dosage, it is lacking in accuracy and tolerance bears no direct relation to weight. While in the acute infections of mice and rats there do not enter the modifying factors of pathological changes and lesions of the fixed tissues and organs together with debilitation and poor general conditions which are so important in the treatment of natural trypanosome diseases, yet these changes can be quite successfully duplicated by employing experimental infections of rabbits with such virulent organisms as *Tr. brucei*, *Tr. gambriensi* and *Tr. equiperdum.* Indeed, in the rabbit these modifying factors may be created more or less at the will of the investigator and may reach a greater degree of severity than ordinarily encountered in natural infections.

Animal experimentation in trypanosomiasis has, therefore, a very direct and valuable application to the treatment of natural trypanosome diseases.

Indeed, the application is even wider, because with some trypanocidal medicaments, and particularly arsphenamin and neoarsphenamin, the results are applicable for the laying down of principles for the treatment of human syphilis.

Confusion Regarding Dosage, Intervals and Route of Administration of Trypanocidal Compounds.—In relation to the very important questions of dosage, frequency and route of administration of medicaments in the treatment of trypanosome diseases, experimental chemotherapy furnishes data of direct value and application.

In the treatment of human trypanosomiasis and syphilis the greatest divergence of opinion and practice can be found among experienced physicians regarding the size of the dose, the frequency and route of administration, to mention nothing of the selection of medicament or the question of using two or more medicaments as a combination therapy. Whether the medicaments should be given once a week, twice a week, daily for three doses or daily for six doses, etc., are questions of debate and each plan has been endorsed by various physicians so that great confusion exists regarding the best course to pursue. Of course, a large number of factors require consideration, among which may be mentioned the questions of toxicity, the stage of the disease, the route of administration, the rate of absorption and elimination of the medicament and the rapidity with which a compound is rendered therapeutically active in the body after administration, granting that pentavalent compounds like atoxyl first require reduction and that trivalent compounds like arsphenamin and neoarsphenamin are not of themselves trypanocidal or spirocheticidal but first require oxidation in the body.

The therapy of relapses in both trypanosomiasis and syphilis of human beings constitutes the greatest problem in treatment. The treatment of acute early infections offers no difficulties; the principles are fairly well understood and the results generally excellent. But in relapses a chronic infection is present in which the questions of possible acquired resistance or fastness of the parasites to drugs require consideration, as likewise the even more important modifying factors of penetrability of the drug into the diseased tissues, the toxicity of the medicament for already damaged tissues and organs and the general health and condition of the subject. But here again these conditions may be duplicated in the lower animals and especially the rabbit, both in trypanosomiasis and syphilis. It requires, however, that the experimental chemotherapeutist shall treat each rabbit as an entity rather than as one of a group since treatment should be planned for each individual as a separate problem.

The employment of rabbits in experimental trypanosomiasis and syphilis affords not only a means for selecting the best medicament and deciding on the questions of size and frequency of dosage, but also the route of administration, concerning which much difference of opinion exists among physicians. With these general considerations in mind, I have conducted a large series of investigations for the purpose of eliciting data of practical value regarding dosage, frequency and route of administration of the more important trypanocidal compounds in the treatment of acute and chronic trypanosomiasis. While these results are more directly applicable to the treatment of trypanosome diseases of man and the lower animals, I believe that they are likewise applicable to the treatment of syphilis and especially with the organic arsenical compounds of the arsenobenzol group, since a fairly close relationship between their trypanocidal and spirocheticidal properties has been demonstrated.

Methods of Administration in Relation to the Treatment of Acute Trypanosomiasis of the Rat.—In conducting these experiments white rats were infected with *Tr. equiperdum* and treatment started 24 hours later according to the technic of my method described in Chapter X. These conditions were most favorable for the medicament, as the blood invasion had just begun when treatment was instituted.

Results with Arsphenamin.—The single minimal curative dose of the arsphenamin employed was 0.004 gm. per kilogram of weight corresponding to 0.28 gm. per 70 kilograms. This amount yielded 100 per cent. cures; 100 per cent. cures were also observed with the following:

3 daily intravenous injections of 0.003 gm. per kilo.
6 daily intravenous injections of 0.002 gm. per kilo.
5 twice weekly intravenous injections of 0.0026 gm. per kilo.
6 weekly intravenous injections of 0.0026 gm. per kilo.

In other words, when three daily doses were given, the amount required was three quarters of the single minimal curative dose; 6 daily doses required one half the minimal curative amount in each while in weekly and twice weekly methods of administration multiple doses of two thirds the single minimal curative dose were required.

Results with Neoarsphenamin.—The minimal curative dose of the neoarsphenamin employed was 0.006 per kilogram; this single dose yielded 100 per cent. cures. Similar results were observed with the following:

3 daily intravenous injections of 0.005 gm. per kilo.
6 daily intravenous injections of 0.0045 gm. per kilo.
4 twice weekly intravenous injections of 0.005 gm. per kilo.
8 weekly intravenous injections of 0.005 gm. per kilo; cured 50 per cent. of animals.

It will be noted that, when multiple doses of neoarsphenamin were administered, at least three quarters of the single minimal curative dose was required when the injections were given on three to six days consecutively or twice weekly. When the injections were given once per week, almost the minimal curative dose was required because the effects of the compound were much shorter than observed with arsphenamin and the few organisms escaping destruction multiplied so rapidly as to destroy the lives of about 50 per cent. of the animals before the next injection could be given.

Results with Atoxyl.—The minimal curative dose of the compound employed in this study was 0.060 gm. per kilogram, the maximum tolerated dose being 0.150 gm. As a general rule, the administration of 0.060 to 0.080 gm. per kilogram resulted in 100 per cent. of cures. Multiple doses yielded the following results:

3 daily intravenous injections of 0.040 gm. per kilo.
6 daily intravenous injections of 0.030 gm. per kilo.
4 twice weekly injections of 0.050 gm. per kilo; cured about 40 per cent. of animals.
6 weekly injections of 0.050 gm. per kilo.; cured about 20 per cent. of animals.

These results have shown that best results with divided doses of atoxyl were observed with the 3 to 6 daily dose plan, the amounts required being about one half of the single minimal curative dose. With the twice weekly and weekly plans the surviving organisms were frequently able to proliferate so rapidly as to destroy the rats before the succeeding dose was due.

A general result of this study was to show that in the treatment of acute trypanosomiasis of the rat in which the death of untreated controls occurred on the fifth to sixth day after inoculation with *Tr. equiperdum*, best results with divided doses were observed when the treatments were given for three to six days consecutively. *If these results have an application to the treatment of acute trypanosomiasis and syphilis of human beings in the earliest stages of these diseases, it would be to indicate that, when a single curative dose cannot be given, best results occur when smaller doses are given intensively, that is, by intravenous injection for three to six days in succession.*

Methods of Administration in Relation to the Treatment of Chronic and Relapsing Trypanosomiasis of the Rabbit.—With rabbits information of greater practical value relating to route of administration, frequency and size of dosage may be obtained since rabbits infected with *Tr. brucei*, *Tr. equiperdum* or *Tr. gambiensi* develop chronic lesions of the skin, face, genital organs, etc., bearing a close similarity to the lesions of natural trypanosome

diseases of human beings and the lower animals. In rabbits, however, the disease pursues a more rapid course than in human beings, but they present the same problems of penetrability or medicament, fastness of parasite, general debility of host, etc., as encountered in natural infections.

In conducting these therapeutic experiments, treatment was begun about three weeks after infection and at a time when the majority of animals showed well-advanced tissue changes, loss of weight and general debility. After treatment the animals were kept under observation for four months or longer and relapses treated as they developed.

The single curative dose of *arsphenamin* by intravenous injection for chronic *Tr. equiperdum* infections of rabbits is about 0.020 gm. per kilogram. Smaller doses suffice for dissipating the lesions and symptoms for variable periods, but relapses generally occur some weeks later. This dose corresponds to 1.2 gm. per 60 kilograms or about 130 pounds, the average weight of an adult.

With divided doses best results were observed when the treatments were given daily for at least three days in succession. With this plan three daily doses of about 0.010 gm. per kilogram were sufficient. When the compound was administered twice weekly, the amount required was from 0.010 to 0.015 gm. per dose for at least two to four doses. When given once a week, almost the full dose was required. Rabbits treated with a dose of 0.010 gm. per kilogram once a week frequently relapsed after three to four doses; the weekly injection of 0.015 gm. per kilo for at least three consecutive weeks generally results in complete cures.

In the treatment of relapses larger amounts were required. Rabbits relapsing two to seven weeks after the injection of 0.005 gm. per kilogram required single doses as high as 0.030 gm. per kilogram to effect a complete cure, or at least three daily doses of about 0.015 gm. per kilogram.

One result of these tests was to show that, when single large doses were contraindicated on account of the extreme loss in weight and debility of the animals, best results were secured by giving smaller amounts at daily intervals for two or three doses; next best results were observed with twice-weekly injections. Under these conditions, the effects of a dose had hardly worn off before re-enforced with the succeeding dose and in this manner sterilization was accomplished without signs or symptoms of toxicity.

Similar results were observed with *neoarsphenamin*, except that larger amounts were required. However, the weekly injections were less successful; likewise, the twice-weekly plan of treatment. Neoarsphenamin is eliminated more rapidly than arsphenamin; at least, its trypanocidal effects are of shorter duration. Daily doses yield excellent results, but with weekly doses surviving trypanosomes are able to proliferate rapidly unless relatively large amounts are given.

The single curative dose for rabbits is about 0.030 to 0.040 gm. per kilogram. Smaller amounts were sometimes curative, but relapses occurred in some animals. Three daily doses in succession of 0.015 gm. per kilogram were generally curative; likewise the twice-weekly injection of 0.020 gm. per kilogram for at least three doses. When the injections were given once a week, doses of 0.010 gm. per kilogram were generally followed by relapses; doses of 0.020 to 0.030 gm. were generally required for effecting complete cures.

Relapses required larger amounts as was found with arsphenamin. Rabbits relapsing three to nine weeks after the injection of 0.005 to 0.010 gm. arsphenamin or neoarsphenamin per kilogram, required single doses of about 0.040 gm. neoarsphenamin for permanent cures or three to four daily doses of about 0.015 to 0.020 gm. per kilogram.

The results observed with *atoxyl* were not so satisfactory, as many animals succumbed before the experiments were completed. The single curative dose by intravenous injection was about 0.050 to 0.080 gm. per kilogram, but these amounts frequently produced fatal intoxication in view of the debilitated condition of the animals. The advantages of smaller doses repeated at close intervals were strikingly apparent; daily injections of 0.020 gm. per kilogram for three to five doses in succession were generally curative, with only exceptional deaths due to toxicity. When given twice weekly at least 0.040 gm. per kilogram was required for three to six doses. Weekly injections generally failed, unless the amount given approached in size the single curative dose which was frequently toxic.

Sulpharsphenamin by subcutaneous or intramuscular injection yielded splendid results. The single curative dose varied from 0.020 to 0.040 gm. per kilogram. Daily doses of 0.010 gm. per kilogram yielded good results when at least three were given in succession. Twice-weekly injections of 0.020 gm. per kilogram and weekly doses of 0.030 gm. per kilogram yielded results equal in curative activity to those observed with arsphenamin and very much better than with neoarsphenamin. In these amounts there were no symptoms of intoxication and in the majority of instances no local reactions at the site of injection beyond small areas of temporary induration. The therapeutic effects of a dose were slightly more prolonged than observed with arsphenamin and much more than with neoarsphenamin, due, probably, to slower absorption and elimination.

Rabbits commonly relapsed after single doses of 0.005 to 0.010 gm. per kilogram. Treatment of these required larger amounts, the single curative dose being about 0.040 gm. per kilogram or three to four daily consecutive doses of 0.015 to 0.020 gm. per kilogram.

Excellent results were likewise observed with *tryparsamid*. The single curative dose by intravenous injection was about 0.4 to 0.6 gm. per kilogram of weight; by intramuscular injection the curative dose was practically the same (0.5 to 0.6 gm. per kilogram).

Three daily intravenous or intramuscular injections of 0.2 gm. per kilogram were generally curative, relapses occurring only twice in a series of six animals; three daily doses in succession of 0.1 gm. each dissipated the lesions temporarily, but relapses generally followed about four weeks later. Twice-weekly doses of 0.3 gm. each for two to five injections were curative; with 0.2 gm. per kilogram relapses occurred. Weekly injections of 0.3 for two to six weeks were likewise curative.

Relapses following previous treatment with atoxyl, arsphenamin and neoarsphenamin generally required a single dose of about 0.6 gm. per kilogram or four daily consecutive doses of 0.2 gm. per kilogram.

With tartar emetic single doses required as much as 0.020 to 0.040 gm. per kilogram by intravenous injection and many rabbits succumbed a few days later as the result of toxic effects. The maximum tolerated dose for healthy normal rabbits was about 0.050 to 0.060 gm. per kilogram, but tolerance was reduced by chronic trypanosomiasis. The advantage of giving smaller doses at frequent intervals was strikingly shown in these experiments. Three to four doses of 0.015 to 0.020 gm. per kilogram by intravenous injection daily in succession produced complete cures in most instances; occasionally an animal relapsed, but none were lost by toxic effects. Twice-weekly injections of 0.020 to 0.040 gm. per kilogram were likewise generally successful when at least three doses were given. Weekly doses generally failed unless almost the single curative dose was given, in which case many animals died within a few days of toxic effects.

Principles of the Chemotherapy of Acute and Chronic Trypanosomiasis.—These experiments have shown that in the treatment of acute trypanosomiasis single large curative doses may be given with success, because tolerance has not been greatly reduced by infection. Since this practice is not usually permissible in the treatment of human beings, the administration of smaller amounts at daily intervals or at intervals of two or three days for several doses in succession have yielded best results. Under these conditions there does not appear to be accumulative toxic effects and the parasites are attacked vigorously over a short period of time with rapidly succeeding doses. "Hitting hard" for a brief period has yielded better results in acute infections than milder treatment by longer intervals between doses, and owing to the good general condition of the subjects the danger of toxic effects is reduced to a minimum.

In the treatment of chronic infections single large doses may produce toxic effects owing to the reduced vitality of the subjects. This is especially true of compounds with relatively low chemotherapeutic indices, that is, the minimal curative doses being but two to four times less than the tolerated or toxic amounts. In chronic infections the administration of smaller doses, at intervals of one to three days for several doses in succession, have yielded best results. Under these conditions the effects of one dose have hardly worn off before the parasites are attacked by a succeeding dose and rapid elimination tends to prevent cumulative toxic effects. When compounds are given intramuscularly, such as atoxyl, tryparsamid and sulpharsphenamin, the doses should be a little larger than by intravenous injection, but the intervals may be lengthened to three or four days or even a week.

Repeated small doses, administered at intervals sufficiently close that the effects of the preceding dose have not entirely disappeared before the succeeding dose is given, permits the compound to penetrate the chronic inflammatory exudates, is especially useful when the general condition of the host is poor and debilitated, is least likely to produce toxic effects and is the method of choice in the treatment of chronic infections. Under these conditions the proper spacing of the injections is almost as important as the number and size of the doses. I

believe that these principles apply in the treatment of acute and chronic syphilis as well as in acute and chronic trypanosomiasis, but further discussion of the subject in reference to syphilis with similar experiments in spirochetic infections of rabbits is given in Chapter XXIX.

A combination of two or more trypanocidal agents may be more effective in the treatment of African trypanosomiasis or infections of the lower animals, than treatment with a single compound, but I shall discuss this phase of the chemotherapy of trypanosomiasis in the succeeding chapter (Chapter XIX).

Intramuscular versus Intravenous Administration of Trypanocidal Compounds.—This route of injection of trypanocidal compounds is a subject of considerable practical importance in the treatment of trypanosomiasis. This is particularly true in Africa where conditions are such that subcutaneous or intramuscular injections are more easily given than intravenous injections and especially on a large scale.

Aside from this question of convenience, however, the subject is of great interest in relation to the principles of chemotherapy and to the treatment of syphilis. In the acute early stages of both trypanosomiasis and syphilis when the parasites are in the lymph and blood, intravenous injections would appear to be indicated, but as soon as the blood has been sterilized, intramuscular or subcutaneous injections may yield better therapeutic results, inasmuch as absorption and elimination are slower, with the result that medicaments are longer retained and may thereby better penetrate inflammatory exudates and reach the parasites in chronic lesions.

The question, therefore, becomes one largely of absorption and elimination and is discussed with more detail in Chapter XXIX, devoted to a discussion of these subjects in relation to the treatment of syphilis. Here may be mentioned the work of Voegtlin and Thompson[1] who found that the excretion of different arsenicals varied after intravenous injection, the directly toxic trivalent oxid compounds showing the slowest rate, the pentavalent arsenicals the fastest, and the arsenobenzene compounds occupying an intermediate position. Elimination of arsenic was found to vary among different individuals of the same species of animals and constituted the chief factor responsible for differences in toxicity and trypanocidal activity. Voegtlin and Smith[2] found that, so far as arsphenamin and neoarsphenamin are concerned, intramuscular injections were just as curative as intravenous injections in the treatment of trypanosomiasis of rats. Unfortunately, however, neither compound can be employed by intramuscular injections in the treatment of human subjects because of the local pain, unless small doses are given in oil, but the principle of the intramuscular route of administration in the treatment of chronic trypanosomiasis and syphilis is based upon sound principles and this route is to be preferred after the blood has been sterilized of parasites, providing intramuscular injections can be borne by patients. This is true of atoxyl, which is largely given in Africa by subcutaneous injection, and it is very much hoped that more powerful trypanocidal agents like sulpharsphenamin will be produced, which may be repeatedly injected subcutaneously or intramuscularly in adequate doses for the treatment of human trypanosomiasis.

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CHAPTER XIX

COMBINATION TREATMENT OF EXPERIMENTAL TRYPANOSOMIASIS AND ITS BEARING UPON THE PRINCIPLES OF CHEMOTHERAPY

A combination of compounds in the chemotherapy of trypanosome and spirochetic infections had always engaged the interest and attention of Ehrlich. Parasites may become resistant to one drug while retaining susceptibility to others. Furthermore, important reactions *in vivo*, probably involving the colloidal state of compounds in the blood and other body fluids, may modify toxicity of the compounds alike for both body cells and parasites. Thus Kolle[1] has recently stated that, contrary to usual impressions and expectations, the simultaneous intravenous injection of a soluble mercurial compound and arsphenamin does not increase toxicity of either compound for the host; on the contrary, toxicity for the host is usually reduced, while parasiticidal activity is actually greater than would be expected from a mere summation of the effects of each compound separately. In this connection it may be mentioned that Browning[2] observed that the treatment with arsacetin and trypan-blue of mice infected with *Tr. brucei* yielded better results than treatment with either compound alone.

Increased Therapeutic Activity of Arpshenamin and Neoarsphenamin in Combination with Mercury.—According to Kolle the toxicity of mercury is reduced in mixtures with arsphenamin because the latter serves, so to speak, as a protective colloid for the colloidal mercury. Arsphenamin derivatives containing a formaldehyd sulphoxylate group are to be preferred in his opinion, because they possess points of attack for the oxidizing mercurial compounds in the formaldehyd group and thereby effect a derivation of the mercury from the arseno group. Since the mercurial preparations enter into a reciprocal action with the arsphenamin compound, whereby the latter is partly changed, and as nearly all mercurials possess an oxidizing power, it is well to employ mercurials of weak oxidizing activity, since arphenamin is a strongly reducing compound and it is advisable in the mixtures to keep the arseno group as intact as possible. From this standpoint mercuric chlorid was found less suitable by Kolle than a new mercurial compound designated as "novasurol."

In this connection I may summarize the experimental work of Neisser[3] in the treatment of syphilis of monkeys with mercury salicylate and atoxyl, although, since these were not always injected together but separately (although at the same time), the effects are not exactly similar to the administration of two compounds in the same solution. Four animals, injected subcutaneously with 0.040 gm. of mercury salicylate and 0.6 gm. of atoxyl per animal (2 to 6 kilos) at the time of inoculation with virus, escaped infection. Of nine additional monkeys injected with 0.040 gm. of mercury salicylate and 0.2 gm. of arsacetin, eight escaped. Of eighteen animals treated with 0.04 to 0.06 gm. of the salicylate and 0.2 to 0.9 gm. of atoxyl after the development of primary lesions, twelve and possibly fourteen, were cured. Of ten monkeys treated with 0.008 to 0.076 gm. of mercuric arsanilate (Uhlenhuth's atoxyl-mercury compound) per kilogram, six were probably cured (in three re-inoculation was negative indicating no cures and in one, spirochetes persisted in the lesions).

Toxicity and Trypanocidal Activity of Mixtures of Solutions of Arsphenamin, Neoarsphenamin and Mercurophen.—As mentioned in Chapter XIV, organic compounds of arsenic and mercury have not so far proven of much value in the treatment of trypanosomiasis, but I have considered it worth while to continue investigation along these lines by studying mixtures of solutions of arsphenamin, neoarsphenamin and various soluble salts of mercury. In chronic syphilis especially, a system of combination treatment with both arsphenamin and a mercury compound administered simultaneously may yield better results than either alone, as discussed in Chapter XXX.

Solutions of most salts of mercury are not suitable for mixture with arsphenamin and neoarsphenamin. Solutions of the bichlorid, benzoate, cyanid, oxycyanid, succinimid and cacodylate, when added to alkalinized solutions of arsphenamin or solutions of neoarsphenamin in water, yield dark, yellowish-green mixtures with the formation of precipitates. Solutions of mercurophen (sodium oxy-mercuri-ortho-nitro phenolate) in water added to solutions of arsphenamin or neoarsphenamin are, however, usually perfectly satisfactory for at least twenty-four hours and for longer periods if sealed in vacuum; some lots of mercurophen, however, may produce slightly opalescent mixtures. Linsser however, administers intravenously the dark greenish mixture of mercuric chlorid and neoarsphenamin in the treatment of syphilis (see page 353).

Trypanocidal tests with these mixtures have yielded very interesting results. White rats infected with *Tr. equiperdum* were employed according to the technic of my method. In one experiment an alkalinized solution of arsphenamin was trypanocidal in dose of 0.003 gm. per kilo; when mixed with 0.002 gm. of mercurophen per kilo, the trypanocidal dose of the arsphenamin was reduced to 0.0015 gm. per kilo; and when mixed with 0.001 gm. mercurophen, the dose was still further reduced to 0.001 gm. Mercurophen itself had no demonstrable trypanocidal effects in the amounts employed, namely, 0.002 and 0.001 gm. per kilo. In an experiment with neoarsphenamin, the smallest trypanocidal dose was found to be 0.008 gm. per kilo. When 0.002 gm. of mercurophen per kilo was added and injected, the trypanocidal dose of the neoarsphenamin was reduced to 0.005 gm. per kilo; when 0.001 gm. of mercurophen was added, the trypanocidal activity of the neoarsphenamin was reduced to 0.004 gm. per kilo.

Additional experiments with other lots of arsphenamin and neoarsphenamin yielded essentially similar results and have shown that the trypanocidal activity of arsphenamin and neoarsphenamin is sometimes, but not always, enhanced by the addition of sub-toxic amounts of mercurophen. Somewhat similar results have been observed in the treatment of rabbit syphilis, described in Chapter XXX.

The maximum tolerated dose of mercurophen by intravenous injection into rats is approximately 0.008 gm. per kilo; the doses employed, therefore, were one-fourth and one-eighth this amount. Toxicity tests, conducted by injecting rabbits intravenously twice a week with 0.020 gm. arsphenamin and 0.001 gm. mercurophen per kilo, have shown no ill effects after as many as eighteen of the combined injections; these amounts corresponded to 1.2 gm. arsphenamin and 0.060 gm. (1 grain) of mercurophen respectively, per 60 kilograms or the weight of an adult human being.

Trypanocidal Activity of Mixtures of Solutions of Arsphenamin and Flumerin, and of Solutions of Neoarsphenamin and Flumerin.—As stated in Chapter XXIV *flumerin*, disodium hydroxy-mercuri fluorescin, prepared by White, Hill, Moore and Young, is a remarkable compound from the standpoint of low toxicity, since it contains about 32.5 per cent. of mercury and is tolerated by rats and rabbits in amounts of 0.030 to 0.040 gm. per kilogram by intravenous injection. *Mercurophen* contains from 46 to 50 per cent. mercury and is tolerated in dose of about 0.006 to 0.008 gm. per kilogram, so that while the mercury content of *flumerin* is 1.5 times less than in mercurophen, its toxicity is about 5 times less, showing that in terms of pure mercury *flumerin* is considerably less toxic than mercurophen.

Mixtures of 0.5 per cent. disodium solutions of arsphenamin and 2 per cent. solutions of *flumerin* in proportion of 10:1 showed no color changes or separation of mercury in six hours at room temperature, but after standing eighteen hours metallic mercury had separated out from the solution. Mixtures of 10 per cent. solutions of neoarsphenamin in water with 2 per cent. solutions of flumerin in proportion of 2:1 likewise showed no color or other visible changes in periods up to six hours at room temperature, although after eighteen hours some metallic mercury had separated from the solution. It was easily possible, therefore, to mix solutions of flumerin and arsphenamin or neoarsphenamin and administer by intravenous injection any time during the first six to ten hours.

In a series of trypanocidal tests employing *Tr. equiperdum*, flumerin was found without appreciable curative activity in single doses as high as 0.030 gm. per kilo administered twenty-four hours after infection. However, when added in amounts of 0.005 to 0.010 gm. per kilogram to solutions of arsphenamin and neoarsphenamin, the trypanocidal activities of the latter were slightly increased. For example, a dose of 0.001 gm. arsphenamin per kilo restrained the appearance of trypanosomes in the peripheral blood for four days after inoculation, but did not prove curative; this dose plus 0.010 gm. of flumerin per kilo, however, restrained the development of trypanosomiasis for ten days. Neoarsphenamin in dose of 0.002 gm. per kilogram restrained trypanosomes for eight days, but was not curative; this dose plus 0.010 gm. of flumerin restrained the infection for sixteen days. These results are, therefore, similar to those observed with mercurophen and indicate that amounts of flumerin without demonstrable trypanocidal activity are capable of appreciably increasing the trypanocidal activity of arsphenamin and neoarsphenamin. The addition of 0.001 to 0.002 gm. of flumerin per kilogram of weight to the doses of arsphenamin and neoarsphenamin did not increase trypanocidal activity and, curiously, a dose of 0.020 gm. of flumerin added to the solutions of arsphenamin and neoarsphenamin was likewise without appreciable effect; indeed, this dose apparently increased the severity of infection probably by reducing resistance through toxic effects upon the host.

Trypanocidal Activity of Arsphenamin and Neoarphenamin in Combination with Tartar Emetic, Sodium and Potassium Tartro-bismuthate, Tryparsamid, Mercurophen, Trypan-red and Trypan-blue.—In view of the results of these experiments, showing that mixtures of arsphenamin and neoarsphenamin with the two organic mercurial compounds mercurophen and flumerin, sometimes resulted in producing a greater degree of trypanocidal activity than a summation of curative effects of the respective drugs alone, additional experiments have been conducted with mixtures of arsphenamin and neoarsphenamin with other trypanocidal agents as tartar emetic, sodium and potassium tartro-bismuthate, trypan-red and trypan-blue.

My purpose was to use each drug in about one-fourth of its trypanocidal dose in order to determine if mixtures of two would enhance curative activity. In some instances the development of trypanosomiasis was delayed, and especially by arsphenamin, neoarsphenamin and tryparsamide, but cures were not produced by the doses employed. Mixtures of arsphenamin or neoarsphenamin with tartar emetic, sodium and potassium tartrobismuthate and trypan-red did not show any increase of trynanocidal activity; indeed, the addition of these compounds to solutions of arsphenamin and neoarsphenamin actually hastened the development of trypanosomiasis with lethal results. The only explanation to be offered at this time is that the toxicity of these compounds reduced the resistance of the rats and thereby favored the development of trypanosomiasis.

The addition of tryparsamid, mercurophen and trypan-blue, however, usually resulted in slightly increasing the trypanocidal effects; with tryparsamid these results may have been due to a summation of trypanocidal activities, but with mercurophen and trypan-blue this explanation hardly suffices since the compounds alone in the doses employed were totally without demonstrable curative activity.

Voegtlin and Smith have observed a complementary action of arsenical and antimony compounds and recently Myers and Corbitt[4] have reported that the administration of neoarsphenamin to rats in subeffective dose (0.005 gm. per kilo) by intraperitoneal injection twenty-four hours after treatment with sodium and potassium tartrobismuthate, resulted in prolonging the lives of some animals beyond the time noted with neoarsphenamin alone; the tartrobismuthate alone was without curative effects and the trypanocidal tests were conducted after the method of Voegtlin and Smith.

Chemotherapeutic Significance of Combination Therapy.—The subject of combination therapy is, therefore, full of theoretical and practical interest and importance. Not only may the administration of two compounds of

totally different origin increase parasiticidal effects by a mere summation of the curative activities of each alone, but, as shown by Kolle, it would appear that one compound may enhance the parasiticidal effects of another by physico-chemical changes.

Of course, combination therapy has been used empirically for many years, as, for example, atoxyl and tartar emetic in human trypanosomiasis and arsphenamin or neoarsphenamin with mercury in the treatment of syphilis. The synergistic effects of two or more drugs is the basis for the prescription of several drugs acting in the same way as, for example, several purgatives prescribed together may act more efficiently than any one given in quantity equal to all of them. Likewise a mixture of morphin and chloral is sometimes more efficient than either administered alone in larger dose. From the standpoint of the practical chemotherapy, the whole subject is of considerable importance and especially in relation to the treatment of trypanosomiasis and syphilis.

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CHAPTER XX

CHEMOTHERAPY OF TRYPANOSOME DISEASES OF MAN AND THE LOWER ANIMALS

It is not my purpose to mention here the very large number of different substances that have been advocated from time to time in the treatment of trypanosomiasis of human beings and the lower animals; excellent reviews are to be found elsewhere.[1] Rather shall I confine myself to a discussion of the subject from the viewpoint of the pathological changes produced in these diseases and the knowledge gained from more recent chemotherapeutic researches in experimental trypanosomiasis. Regarded from these standpoints, I believe the principles of the treatment of trypanosomiasis of both human beings and the lower animals are essentially similar to those applicable in the treatment of syphilis in its acute and chronic stages, and that a great deal which we have learned regarding the treatment of the latter disease may be applied in principle to the treatment of trypanosomiasis.

Etiology of Trypanosomiasis of Man.—Fortunately, only three or four species of the large number of known pathogenic trypanosomes are capable of infecting human beings. Presumably none of those producing disease in horses, mules, cattle, sheep, camels and other of the lower animals appear capable of infecting man; positive statements, however, cannot be made, because there is no conclusive evidence that human beings are absolutely immune to *Tr. evansi*, *Tr. brucei*, etc. Indeed some investigators regard *Tr. rhodesiensi*, known to produce a form of African trypanosomiasis, as being identical with *Tr. brucei*, the cause of "nagana disease" of horses. It may be stated, however, that trypanosomes are not found in human beings except in disease, that is, non-pathogenic or saprophytic forms are not encountered analogous to *Tr. lewisi* of rats, *Tr. rotatorum* of frogs, etc.

The best known trypanosome disease of man is *African trypanosomiasis*, designated and more familiarly known as "African *sleeping sickness*," owing to the torpor of the later stages of the infection. At least two very closely related trypanosomes are known to be the cause of this disease, namely, *Tr. gambiensi*, transmitted by the tsetse fly (*Glossina palpalis*), and *Tr. rhodesiense*, transmitted by the fly (*Glossina morsitans*). Recently Macfie has described another subspecies: *Tr. nigeriense*, transmitted by *Glossina tachinoides*. The disease, however, produced by these three trypanosomes is essentially similar in its pathological changes and symptoms, although variations in virulence are encountered in different localities as modified by epidemiological factors. The exact relationship of *Tr. rhodesiense* to *Tr. gambiensi*, however, has not been established. When the former is passed through the rat it presents certain morphological differences, although in human blood it is morphologically indistinguishable from *Tr. gambiensi*. While the symptoms of *Tr. rhodesiense* infection are similar to those produced by *Tr. gambiensi*, the febrile paroxysms are more frequent, glandular involvements are usually more severe and the disease generally runs a much more rapid course. According to some observers animals immunized against *Tr. gambiensi* are not protected against *Tr. rhodesiensi* and if the latter is not a distinct species, it is at least a very definite variety producing disease of human beings much more difficult to cure.

A third type of human trypanosomiasis is known as "Chagas' disease" caused by *Tr. cruzi*, transmitted by a bug, the *Conorhinus megistus*. This disease has been found in Brazil and Venezuela and is sometimes designated as "American trypanosomiasis." The armadillo is supposed to be the natural reservoir of this trypanosome, 50 per cent. of the animals being infected. In Venezuela the intermediate host is *Rhodnius prolixus*.

More recently Escomel reports the discovery of a human trypanosome in Peru, which Yorke considers a distinct species (*Tr. escomeli*).

The Nature and Pathology of African Trypanosomiasis in Relation to Chemotherapy.—African trypanosomiasis in its early stages is essentially an infection of the lymphatic system and to some extent of the blood; later the tissues are involved and especially those of the central nervous system. In these respects the disease is similar to syphilis in that the lymphatic system is likewise early invaded by *Sp. pallida* accompanied by early spirochetemia with later involvement of the fixed tissues and especially of the central nervous system.

The period of two to three weeks primary incubation in trypanosomiasis, the degree of local irritation of greater or less severity at the site of the bite of an infected *Glossina* noted in some cases, the irregular fever, debility, early polyadenitis, slight anemia and papular eruptions which may continue for months are additional clinical features bearing some similarity to those found in syphilis. The local edemas of human trypanosomiasis, which are likewise characteristic of the experimental disease in rabbits, is a special feature due to the embolic blocking and endothelial hyperplasia of lymphatic channels by the large trypanosomes. Doubtless if *Spirochæta pallida* were larger, similar lesions would develop in human and experimental syphilis more frequently than encountered.

The development in trypanosomiasis of *irido-cyclitis* and *choroiditis* with gradual involvement of other special organs and more particularly the brain, bears a close similarity to the course of syphilis. The psychical and physical symptoms of the former are usually different from those developing in syphilis, because vascular occlusion due to endothelial hyperplasia is frequent, the parasites are larger and the infection usually numerically greater,

resulting in a more rapid development and termination of the disease and such general widespread effects as increasing torpor and physical degradation, headache, general tremors and convulsions, ataxia and terminal mania. In such cases trypanosomes have been found not only in the capillaries of the brain, but in the brain substance itself (cerebral trypanosomiasis), quite analogous in this respect and in the pathologico-histological changes produced to paresis or infection of the brain substance and perivascular spaces with *Sp. pallida*. I have found similar changes in the brains of some rabbits dying of *Tr. equiperdum* infection and both rats and rabbits are apt to die with the tremors and convulsions characteristic of the terminal stage of trypanosomiasis in human beings. In the early stages of African trypanosomiasis the parasites are found in the blood and lymphatic glands more frequently and readily than in the later stages; consequently during these early months treatment is much more effective than in the later stages when widespread and chronic tissue lesions have developed. These tissue changes are principally in the lymphatic tissues throughout the body, in the meninges and possibly in the muscles (myositis with cysts similar to those produced by *Tr. cruzi* in Chaga's disease). As first shown by Mott, practically every case shows congestion of the meninges with extensive meningo-encephalitis and preivascular round-cell infiltrations throughout the meninges, brain and cord with secondary degenerative changes of the nerve-cells analagous to the histological changes in syphilis (especially paresis). The cerebrospinal fluid is increased in quantity, shows an increase of cells (principally lymphocytes), an increase of protein and sometimes the presence of the trypanosomes (first described by Castellani in 1902). Whether or not changes occur in the sugar-content as in syphilis, I cannot state; neither have I been able to find data upon the complement-fixation and colloidal-gold reactions with the spinal fluid in this disease. The presence of trypanosomes in the spinal fluid occurs so frequently that routine examination of this fluid constitutes an aid in diagnosis. Furthermore, from the standpoint of chemotherapy, the indications are to select a trypanocidal compound possessing the properties of penetration into the cerebrospinal fluid following intravenous injection, and in this connection may be mentioned the recent work of Voegtlin, Smith, Dyer and Thompson,[2] who found in rabbits that sulpharsphenamin, tryparsamid and 3-amino-4-oxyphenyl arsenic acid were remedies of superior penetrative power.

All experimental and clinical evidence shows that from the standpoint of chemotherapy, best results are observed when treatment is given during the early stages of the infection when the parasites are in the blood and lymphatic system and before fixed tissue changes have occurred. The same is true of syphilis, only the necessity for early diagnosis, prompt and *vigorous* treatment are still more important in trypanosomiasis because this disease is more acute and runs its course more rapidly. In the chronic stages of human trypanosomiasis fibrosis of the infected tissues, and especially of the lymph-glands where the trypanosomes may rest, likewise adds difficulties in treatment and requires intermittent treatment over long periods of time as in the treatment of chronic syphilis.

Fortunately trypanosomiasis does not appear to be transmitted to offspring as frequently as in syphilis. According to Greggio, human trypanosomiasis is not, as a rule, transmitted, although the abortion rate is increased from the normal 7 per cent. in the Congo natives to 31.7 per cent., and the infant mortality for 29 to 50 per cent. Among the lower animals I have frequently observed abortion of rats, guinea-pigs and rabbits, when infected with *Tr. brucei* and *Tr. equiperdum*, but have not at the present time been

able to find trypanosomes in the foetuses. Koch, from observation in Ugunda, thought that human trypanosomiasis may be transmitted by the male during coitus. Dourine (*Tr. equiperdum*) of horses is known to be transmitted in this way, but the evidence in support of transmission of human trypanosomiasis is not conclusive.

Chemotherapy and General Treatment of African Trypanosomiasis (Sleeping Sickness).—Even though the lower animals infected with *Tr. gambiensi* or *Tr. rhodesiense* may be cured by the intravenous injection of a single dose of any one of several trypanocidal compounds, this result is not ordinarily obtained in the treatment of human trypanosomiasis. If human beings could be treated early enough, the chances for such success would be much greater; but unfortunately the infection is usually well established before the natives seek treatment. In syphilis the development of the primary lesion usually excites the majority of even the most careless and ignorant to seek some sort of medical aid, but the local irritation produced by the bite of the infected tsetse fly presents nothing that is characteristic and even blood examinations may be negative in the early stages until the trypanosomes have multiplied considerably and invaded the lymphatic glands. As is true of syphilis, *the earlier treatment is instituted in trypanosomiasis, the better are the chances for success;* but in practice, the difficulties of making an early diagnosis are much greater in trypanosomiasis than in syphilis.

Indeed Ouzillean and Lefrou have recently stated that the treatment of the late or sleeping stage is so unsatisfactory that it resolves itself into preventive or prophylactic treatment only, that is, treatment of the first stage to prevent trypanosomanic invasion of the brain and meninges.

A large number of drugs and principally arsenical compounds, have been administered orally in the treatment of trypanosomiasis. The results have been very poor. Absorption is too irregular, the tolerated doses by this route are too small and the disease progresses too rapidly to justify this kind of treatment if it is possible to employ the intravenous, intramuscular or subcutaneous routes of administration. In the treatment of acute syphilis, the old practice of relying upon the administration of mercury and iodid by mouth has been largely abandoned for the same reasons, but in trypanosomiasis the progress of the infection is ordinarily so much faster, that oral administration is even less efficacious. After the trypanosomes have been apparently destroyed, drugs of the arsenical and antimony group may be given by mouth during the year or preferably two years of "watchful waiting" to determine if cure has been accomplished, just as may be practiced in the treatment of syphilis, but otherwise oral administration has no place in the treatment of trypanosomiasis.

The specific treatment of trypanosomiasis of the human being requires the subcutaneous, intramuscular or intravenous administration of trypanocidal agents, just as is true of the treatment of experimental infections. In practice among the African natives intravenous therapy may be very difficult, but it and intramuscular therapy occupy the first positions of choice. As will be shortly discussed, a form of combined intravenous and subcutaneous therapy is probably best. Drugs have also been injected intraspinally (subthecal) in the treatment of the later stages when trypanosomes are to be found in the cerebrospinal fluid, and under certain conditions it would appear that this route of administration has certain advantages.

Since Lingard introduced arsenious acid, Manson sodium antimony tartrate and Broden and Rodhain tartar emetic in the treatment of trypanosomiasis, a large number of organic and inorganic arsenical and antimony compounds have been introduced. Likewise many other substances have

been employed, but it is the consensus of opinion that arsenical and antimonyl compounds have proven most efficacious.

Treatment with Atoxyl.—Of the arsenical compounds, *atoxyl* has been mostly employed. It has the advantage of being administrable by subcutaneous injection and possessing a fairly high trypanocidal activity. A distinct disadvantage is its affinity for the optic nerve and the tendency for producing toxic amblyopia, and the drug should not be used at all if the eye is involved by the trypanosome infection. Atoxyl is more useful in the treatment of the first stages of trypanosomiasis than in the "sleeping sickness stage" although contrary to current opinion, it may bring the disappearance of typanosomes in the cerebrospinal fluid with a decrease of cells and protein. When administered intravenously atoxyl is also capable of exciting reactions of fever, cutaneous eruptions, etc., somewhat similar to those produced by arsphenamin. Atoxyl, however, is not usually responsible for the epileptiform convulsions of trypanosomiasis; rather these are caused by the trypanosomes. Ouzilleau and Lefrow for example, claim that these effects may be due to the disease rather than to the drug and that in the first stage they do not develop at all if care is taken not to give more than 1.5 gm. of atoxyl in a single dose. In the late or sleeping stage, evidences of neurotropism are more apt to develop, not only with atoxyl, however, but likewise with neoarsphenamin and other arsenical compounds indicating that the organotropism (neurotropism) of arsenical compounds at this time is greater than their parasitropism due to the pathological changes of the brain and meninges.

Recently a French Commission on the treatment of sleeping sickness[3] have reported favorably on the use of atoxyl in doses of from 0.5 gm. to as much as 1.0 gm. for adults interspaced with the intravenous injection of tartar emetic in dose of 0.08 to 0.1 gm. Ouzilleau and Lefrou[4] and Lefrou[5] have found that this mixed treatment with atoxyl and tartar emetic not only influenced the early stages of trypanosomiasis but the late stages as well with a gradual improvement of the spinal fluid after 5 to 6 injections of both medicaments. More recently Blanchard and Laigret[6] have reported that in the first stage of trypanosomianis six subcutaneous injections of atoxyl in dose of 0.02 gm. per kilogram of weight at two week intervals, yielded excellent results and especially with regard to the permanent removal of trypanosomes from the circulating blood. Intravenous injections of tartar emetic between the atoxyl treatments aided the results.

Treatment with Arsacetin, Arsenophenylglycin and Amino-oxyphenyl Arsonate.—Arsacetin and *arsenophenylglycin* were at one time employed, but both have proven too toxic for repeated administration. In effective doses they possess a high degree of neurotropism, or affinity for nerve tissue in general and the optic nerve in particular. Pentavalent arsenical compounds generally possess a higher degree of penetration of the arsenic into nerve tissue, followed by injury, than is the case with the trivalent arsenobenzenes. Blanchard and Lefrou[7] have recently reported good results from the use of *amino-oxy-phenyl arsonate*[189] and as previously stated Voegtlin and his associates found that this substance (the sodium salt of 3-amino-4-oxyphenyl arsonic acid) had superior penetrative power for the spinal fluid in rabbits.

Treatment with Arsphenamin and Neoarsphenamin.—Trivalent compounds of arsenic, as *arsphenamin* and *neoarsphenamin*, would appear to be more efficacious in the treatment of the early stages since they are readily reduced in the tissues to the trypanocidally active oxids. Both of these compounds possess an extremely high trypanocidal activity in experimental *Tr. gambiensi* infections, and arsphenamin is especially indicated in the treatment of the late stages of the human disease because it penetrates the cerebral tissues and reaches the spinal fluid somewhat more readily than neoarsphenamin. The results observed in the treatment of human trypanosomiasis with these compounds, have not by any means, however, been as good as observed in the treatment of syphilis. Derwish[8] has recently reported that of 29 cases treated about 8 years ago with salvarsan and tartar emetic 13 are still in good condition, 3 are in fair condition and 13 have died.

Treatment with Tryparsamid.—Louise Pearce[9] has reported a thorough study of *tryparsamid* in the treatment of both early and late cases of African trypanosomiasis in the Belgian Congo caused by *Tr. gambiensi.* I have already described, in the preceding chapter, the results of treatment with this pentavalent compound in experimental *Tr. gambiensi* infections observed by Dr. Brown and Dr. Pearce. The experimental studies were so favorable that it is hoped the compound will mark an advance in the treatment of human infections. In the first stages of the disease the intravenous injection of single doses varying from 2 to 5 gm. was usually followed by a peripheral sterilization in from 6 to 12 hours which lasted on an average of from three to six weeks although in one case a relapse occurred 17 days after treatment. Intramuscular injections were even more efficacious in that peripheral sterilization followed in the same manner and persisted on the average for seven weeks after treatment. The general effects were likewise prompt and satisfactory.

A number of early cases were also treated with 4 to 7 doses of 2.0 to 5.0 gm. at weekly or fortnightly intervals. The preliminary effects were good both in regards to clinical

improvement and the disappearance of trypanosomes, but nothing can yet be stated regarding the ultimate results.

Equally encouraging results were observed in the treatment of advanced cases by injections of 1.0 to 7.0 gm. at intervals of 3 to 14 days. In these cases it is necessary to take into account not only the invasion of the cerebrospinal tissues by the trypanosomes and the resulting injuries, but also the chronicity of the disease, the tendency for trypanosomal relapses and the debilitated condition of many patients.

Under tryparsamid treatment trypanosomes disappeared from the blood, lymph-glands and cerebrospinal fluid of the majority of cases and the cells of the spinal fluids were decreased by 57 to 98 per cent. in an average of 5 weeks. The general effects were likewise good in most instances, as shown by a return of the pulse and temperature to normal limits, improvement in weight, physical appearance and habits, etc. Weekly doses of 3.0 to 4.0 gm. appeared to yield just as good results as semi-weekly doses of 2.0 gm. Doses of 3.0 gm. or over should not be given oftener than once a week and longer intervals may be just as good and less likely to produce the disturbances of vision that occurred in 9 advanced cases treated by Dr. Pearce. Too frequent doses to patients suffering with very advanced trypanosomiasis is are practically the only conditions in which tryparsamid produced any untoward effects like dimness of vision.

Lefrou and Ouzilleau[10] and Chesterman[11] have recently reported favorable results in the treatment of African trypanosomiasis with tryparsamid. The latter treated 40 selected cases, the usual treatment being the weekly intravenous injection of 3 gm. in 10 c.c. of sterile water. The compound was found to be less toxic than atoxyl and in 24 cases without previous treatment, the primary results were good, the spinal fluids of all being restored to normal. Chesterman states that successful treatment demands maintaining the maximum for at least six to eight weeks and a total of 3 to 4 gm. per week for the average adult is sufficient. Morgan[12] has recently reported the apparent successful treatment of a case after relapse following the administration of three injections of atoxyl and ten injections of "Bayer 205."

Treatment with Sulpharsphenamin.—In the treatment of the advanced cases of trypanosomiasis, the indications are to employ trypanocidal agents capable of penetration into the cerebrospinal fluid, inasmuch as the cerebral lesions are most severe and destructive. Recently an experimental study by Voegtlin, Dyer and Thompson[13] employing rabbits infected by the injection of *Tr. equiperdum* into the cranial subarachanoid space, showed that sulpharsphenamin, tryparsamid and 3-amino-4-oxyphenyl arsonic acid were remedies of superior penetrative power. Judged upon the basis of the power of the drugs injected intravenously reaching the subarachanoid space of rabbits and destroying trypanosomes, the effectiveness of the various compounds was in the following order: tryparsamid, sulpharsphenamin, 3-amino-4-oxy-phenyl arsonic acid, arsacetin, atoxyl, neoarsphenamin, arsphenamin, silver arsphenamin and "arsenoxid." So far I have found no reports upon its use in the treatment of African trypanosomiasis, but the results of animal tests certainly justify its trial.

Treatment with Tartar Emetic and Other Antimony Compounds.—Next to the arsenicals, compounds of antimony and especially trivalent compounds, have been firmly established in the treatment of trypanosomiasis. *Tartar emetic* is mostly employed but must be injected very carefully and intravenously, as the least amount in the subcutaneous tissues or muscles produces a very painful local reaction. Likewise it must be well diluted to reduce the chances of unpleasant and even dangerous reactions of vertigo, cough, syncope, unconsciousness, etc. Usually the dose is 0.1 gm. dissolved in not less than 100 c.c. of sterile saline solution or, better, 5 to 10 c.c. of a sterile 1 per cent. solution injected slowly and carefully.

Various new antimonyl compounds have been advocated. Of these *sodium antimony thioglycollate* described by Rountree and Abel[14] has yielded excellent results in experimental infections. The cost is low, it apparently does not readily produce antimony fastness or resistance of trypanosomes and it is readily administered by intravenous or intramuscular injection of 5 to 10 c.c. of a 1 per cent. sterile solution. *A special advantage consists of the fact that these amounts may be injected intramuscularly without producing local irritation;* this renders available, therefore, an antimonyl preparation for the treatment of children or under conditions generally in which intravenous therapy cannot be applied.

Among other antimonyl preparations may be mentioned the *sodium salt of p-amino-phenyl-stibinic acid* produced by Breinl and Nierenstein,[15] *anilin antimonyl tartrate* introduced by Laveran[16] and the *sodium salt of acetyl-p-aminophenyl stibinic acid* ("stibenyl") employed by Manson-Bahr.[17] This compound contains 34.3 per cent. antimony and is tolerated by human beings in doses of 0.6 gm. One case of trypanosomiasis was greatly improved by repeated injections with a total administration of 4.5 gm. The compound is regarded as more active than tartar emetic in the treatment of *Tr. rhodesiense* infections which are well known to be more difficult to treat than *Tr. gambiensi* infections.

Recently Van den Branden and VanHoff have reported the use of the *tartro bismuthate of potassium and sodium* ("luatol"), but as mentioned in the preceding chapter this substance has practically no effect in experimental trypanosomiasis and I doubt if it will prove of value in the treatment of African trypanosomiasis.

Treatment with "Bayer 205."—At the present time a new compound prepared by the Bayer Company and designated as "205" is being tried in the treatment of African trypanosomiasis and the successful treatment of quite a number of cases has been recorded. As stated in the preceding chapter its composition is secret. Kleine and Fisher,[18] Stephens and Yorke[19] and Low and Manson-Bahr[20] have recently reported favorably upon the use of this compound in the treatment of African trypanosomiasis. The latter have treated 8 cases of *Tr. gambiensi* infections in Europeans with good results in 7 cases; one case died of cerebral trypanosomiasis. One case of *Tr. rhodesiense* infection was greatly improved but not cured. They advise the intravenous injection of 1.0 gm. dissolved in 10 c.c. of water as a dose once per week for ten weeks, although *therapia magna sterilisans* may be attempted in early cases by an injection on each of three days in succession. The compound, however, may produce nephritis with albumin and casts in the urine and should be used cautiously until more is known of its composition and toxicity.

Treatment of Early Stages of African Trypanosomiasis Caused by Tr. Gambiensi.—With this general review of the various medicaments proposed for the treatment of trypanosomiasis of human beings, I may now briefly summarize plans for the treatment of the various stages of the disease. My suggestions however, are based entirely upon the results recorded in literature and extensive animal experimentation rather than upon personal experience with the disease in Africa. In the early stages, treatment should extend over a period of at least six months with an additional period of observation of at least one to two years. In the following method the doses of the different medicaments are those suitable for adults of both sexes; children should be given proportionate amounts according to weight rather than according to age.

(1) During the first year arsphenamin, in alkalinized solution, in dose of 0.6 gm. or neoarsphenamin in dose of 0.9 gm. by intravenous injection may be given once a week for 12 to 15 injections. If intravenous injections are not possible or convenient the intramuscular injection of tryparsamid in dose of 2.0 gm. dissolved in 10 to 20 c.c. of water, or subcutaneous injections of atoxyl in dose of 0.5 gm. dissolved in 5 c.c. of water, may be given. Intramuscular injections of sulpharsphenamin or "Bayer 205" may be administered instead.

(2) This course may be followed by a rest without specific medication of one month to be followed by:

(3) Tartar emetic intravenously in dose of 5 to 10 c.c. of a sterile 1 per cent. solution every other week for 12 injections (6 months) or sodium antimonyl thioglycollate in the same dose by intravenous or intramuscular injection. Tryparsamid may be given in dose of 2 gm. in 10 c.c. of sterile water intraamuscularly at the same time each alternate week for 12 injections covering a period of 6 months; or atoxyl may be administered instead in dose of 0.5 gm. in 5 c.c. of water by subcutaneous injection every other week for 12 injections.

(4) This completes the first year of treatment at the end of which time the patient should be given a rest of a month or two under observation and subjected to a thorough examination for trypanosomes as follows: (*a*) Repeated examinations of the blood including, if possible, animal inoculation tests conducted by injecting citrated or defibrinated blood intraperitoneally into mice (dose 1 c.c.) or white rats (dose 2 to 3 c.c.). (*b*) Examination of blood for auto-agglutination. (*c*) A thorough examination of the cerebrospinal fluid for cells, protein and trypanosomes (microscopical examination of centrifuged fluid and injection of fluid or sediment into mice or rate in case of negative direct examination). (*d*) If the glands are enlarged, puncture and examination for trypanosomes.

(5) If evidences of infection are still present, the above courses should be repeated, at least in part. A careful watch should be made for relapses throughout the second year and treatment given if relapse occurs.

(6) During the second year, while the patient is under clinical and laboratory observation, it may be well to give some treatment even though apparently free of infection, like atoxyl in dose of 0.5 gm. subcutaneously or tryparsamid in dose of 2.0 gm. intramuscularly twice a month. Medication by oral administration hardly appears worth while, although hexamethylenamin (urotropin) in dose of 0.25 gm. (4 grs.) three times a day may be of some advantage in preventing invasion of the spinal fluid.

Treatment of Late Stage of Trypanosomiasis Caused by Tr. Gambiensi.— Treatment of the late stage of African trypanosomiasis with cerebral symptoms of torpor is complicated by the presence of chronic lesions and the poor general condition of the majority of these cases. Various complications must be taken into account and specific treatment administered as indicated for individual cases.

(*a*) Tryparsamid may be given in dose of 2.0 gm. dissolved in 20 c.c. of sterile water intramuscularly once a week for 12 to 15 weeks. Or sulpharsphenamin in dose of 0.4 to 0.6 gm. dissolved in 5 to 10 c.c. of sterile water by subcutaneous injection once a week for 12 to 15 weeks. Atoxyl has not yielded very good results in this stage of the disease.

At the same time a weekly intravenous injection of tartar emetic in dose of 5 to 10 c.c. of a 1 per cent. solution in sterile water or sodium antimonyl thioglycollate intravenously or intramuscularly in the same dose. The arsenic and antimony should not be given at the same time and especially if reactions follow. If intolerance for the antimonyl compound develops or reactions occur it is well to interrupt the injections for a week or two or reduce the dosage, but the rule should be to give the patient as vigorous treatment as conditions permit.

Arsphenamin in dose of 0.6 gm. or neoarsphenamin in dose of 0.9 gm. may be given intravenously once a week instead of tryparsamid or sulpharsphenamin, but the latter two have the advantages of being administrable by intramuscular or subcutaneous injection and possibly furnish arsenic in a form more rapidly penetrating to the cerebrospinal fluid than arsphenamin. Of the two latter compounds, arsphenamin is to be preferred although it is more toxic, more apt to produce reactions and more troublesome to administer.

(*b*) The above course may be lengthened or shortened as indicated by circumstances. Careful examinations should be made of the urine for irritation of the kidneys. After a period of rest of 4 to 6 weeks, the course may require repetition, or treatment for the balance of the year may consist of the intramuscular injection of 3.0 gm. of tryparsamid intramuscularly once every 10 to 14 days or 0.5 gm. atoxyl by intramuscular injection once a week.

(*c*) Indications for repetition of the courses of treatment in the second year are based upon physical examinations of the patient and the examination of blood and cerebrospinal fluid mentioned above.

Relapses frequently occur and during the second year it would appear better not to await the development of these but to administer at least two courses of treatment, each consisting of the intramuscular injection of 2.0 to 3.0 gm. tryparsamid every two weeks, alternating with the intravenous injection of tartar emetic in dose of 5 to 10 c.c. of a 1 per cent. solution every two weeks, for a period of 4 months (8 injections of each) followed by one month of rest. Instead of tartar emetic, sodium antimonyl thioglycollate may be used in the same dosage by intravenous or intramuscular injection.

Intraspinal Treatment of Sleeping Sickness.—Eyre,[21] Trout[22] and Marshal[23] have reported good results in the treatment of sleeping sickness following the Swift-Ellis plan for the treatment of paresis and other forms of syphilis of the brain and spinal cord. These observers have injected 0.9 gm. of neoarsphenamin intravenously and ½ to 1 hour later withdrew 20 to 40 c.c. of blood. After allowing the serum to separate over night, spinal puncture was made, spinal fluid allowed to escape and 10 to 20 c.c. of the serum injected. Trout has also employed galyl, atoxyl, tartar emetic, antimony trioxid and other substances instead of neoarsphenamin.

When trypanosomes are found in the cerebrospinal fluid in this stage of the disease, intraspinal therapy would appear indicated at least in those cases which do not respond to intravenous and intramuscular medication. It would appear worth while, however, to add 0.005 to 0.001 gm. of neoarsphenamin to the serum before injection, the technic being exactly the same as described for the treatment of neuro-syphilis in Chapter LXXVI.

Treatment of African Trypanosomiasis Caused by Tr. rhodesiense.—This form of sleeping sickness is encountered in Nyasaland, Rhodesia, Portugese East Africa and other parts of Africa and represents a particularly virulent type of infection tending to run a more rapid course than *Tr. gambiensi* infections.

Atoxyl and tartar emetic are less efficacious unless treatment is begun early and pushed with vigor.

I do not know the value of arsphenamin, neoarsphenamin, tryparsamid and sodium antimonyl thioglycollate in treatment, but on the basis of animal experiments, I believe that these substances possess curative activity for infections with this particular trypanosome. It would appear, therefore, that treatment should be along the same general lines as described above for *Tr. gambiensi* disease. As stated above, Manson-Bahr has recommended particularly acetyl-p-aminophenyl stibiate ("stibenyl") as more efficacious than tartar emetic; this compound is given intravenously in doses beginning with 0.1 gm. for an adult and gradually increased to 0.6 gm.

General Treatment of Trypanosomiasis.—This consists in maintaining the nutrition and weight of the patient. Individuals who are losing weight do not respond well to specific medication and are particularly likely to develop unpleasant reactions interfering with systematic and thorough treatment. Tincture of nux vomica has been recommended. Infected Europeans in the tropics should be removed if possible to cooler climates. Rest and the systematic use of quinin for any concurrent malarial infection should be insisted upon. Fatigue and everything that might depress, should be carefully avoided.

Duration of Treatment of Trypanosomiasis.—Even in the acute early stages there should be a minimum of treatment covering at least one year. Trypanosomes may disappear from the blood and lymph-glands with less—much less—treatment than advised above, but since relapses so commonly occur it is well to over-treat rather than run the risk of under-treating cases, if conditions permit. Patients should be under observation for an additional year and undergo periodic clinical and laboratory examinations analogous to the periodic use of the Wassermann reaction in the treatment of syphilis. The cerebrospinal fluid should be examined at least once or twice and treatment continued until it is restored to normal.

The question of complete cure when treatment is begun in the late stages, is doubtful and probably occurs only in exceptional cases. These cases should be kept under supervision for years and undergo periodic courses of treatment.

Re-infection in Trypanosomiasis.—It is highly probable that the immunity following the cure of African trypanosomiasis is of brief duration and that susceptibility to re-infection exists analogous to re-infection in syphilis when complete cure of the previous disease has been effected. I have not been able to find accurate data upon this point in reference to human beings, but I know that the lower animals can be re-infected with the same strains of trypanosomes about one or two months after the previous infection has been eradicated by specific medication even when, as in rabbits and guinea-pigs, the infection has persisted for 2½ to 7 months. It is not improbable that relapses in human subjects may indeed be true re-infections, since the chances for re-infection by tse-tse flies is so likely in the infested districts of Africa.

Whether or not a case of human trypanosomiasis is immune to super-infection with *Tr. gambiensi* or *Tr. rhodesiense*, as long as he is infected with one of these parasites and uncured, in a manner analogous to the almost complete resistance of the uncured syphilitic to super-infection with *Sp. pallida* after the primary stage of syphilis, I cannot state. On the basis of animal experiments I believe, however, that the immunity developed during treatment is sufficient to protect against super-infection with either *Tr. gambiensi* or *Tr. rhodesiense*. This immunity, however, probably disappears in about one or two months after cessation of treatment with arsenic, antimony or a mixture of these.

Treatment of American Trypanosomiasis (Chagas' Disease).—Unfortunately, there has been very little experimental work done on the treatment of this disease with the more recently discovered arsenical and antimony compounds. Manson-Bahr states "we know of no specific remedy. Arsenicals and antimony have failed in experimental animals. Treatment, therefore, must be on general lines."

As previously stated, the etiological agent is *Tr. cruzi*, also known as *Schizotrypanum cruzi*. The disease occurs in Brazil and Venezuela; possibly also in Peru.

Chagas[24] recognizes acute and chronic types. Children are mostly infected and the parasite produces lesions and symptoms resembling cretinism. Introduced by the bite of infected bugs, the trypanosome multiplies not in the blood, but in the cells of various organs, including the striped muscle, especially of the heart, vertebral muscles, in bone marrow, and other organs and tissues. The parasites then find their way into the blood concurrently with an attack of fever, but usually in small numbers requiring prolonged search. It may be necessary to centrifuge 10 c.c. of citrated blood to find them in smears of the sediment or to inoculate guinea-pigs with 2 to 5 c.c. intraperitoneally, the trypanosomes being subsequently found in the blood, or the developmental forms in the muscles.

Whether or not the trypanosome is susceptible to destruction in human subjects by arsphenamin, neoarsphenamin, tryparsamid or sodium antimonyl thioglycollate cannot be stated; but until suitable animal experiments have been conducted, it would appear worth while to give these substances a trial and especially tryparsamid, sulpharsphenamin and sodium antimonyl thioglycollate which may be administered by subcutaneous or intramuscular injection, since intravenous medication may not be possible in children. The dose of tryparsamid may be 0.050 gm. per kilo (approximately 2 pounds) dissolved in 5 c.c. of sterile water; sulpharsphenamin in dose of 0.005 gm. per kilo dissolved in 2 to 5 c.c. of sterile water and sodium antimonyl thioglycollate in dose of 2 to 5 c.c. of a sterile 1 per cent. solution. Repeated injections are probably required at intervals of 5 to 10 days after the plan described above for the treatment of African trypanosomiasis.

Treatment of Trypanosomiasis of the Lower Animals.—The treatment of trypanosomiasis of the lower animals is not usually undertaken since it is cheaper to destroy the infected animals. In the United States only one trypanosome disease has so far been described, namely, *dourine* or *Tr. equiperdum* infection of the horses of the northwest.

The lesions and symptoms of trypanosomiasis of the lower animals vary with the different parasites. In all, however, the infection is likely to be chronic with infection of the tissues and irregular infection of the blood. In dourine, the tissues of the genital organs are especially involved and diagnosis is aided by the examination of the edema fluid and blood for trypanosomes and by the complement-fixation test.

Practically all that has been written on immunity in human trypanosomiasis and its relation to chemotherapy is applicable to trypanosome infections of the lower animals. Treatment is likewise similar to that described for African trypanosomiasis, except, of course, that the doses of the various medicaments are larger. These doses may be calculated on the basis of kilogram of body weight (approximately two pounds) as follows:

(*a*) *Arsphenamin* in alkalinized solution by intravenous injection in dose of 0.010 gm. per kilo. The dose for a 1600-pound horse would be, therefore, about 5 to 8 gm. dissolved in 500 to 1000 c.c. of water and alkalinized.

(*b*) *Neoarsphenamin,* in dose of 0.015 gm. per kilo dissolved in sterile water. The dose for a 1600-pound horse would be, therefore, about 10 to 12 gm. dissolved in 50 to 100 c.c. of water. Pommrich[25] records the cure of case of dourine in a horse following one dose of neoarsphenamin.

Owing to the cost of these compounds, their administration and especially repeated administration, may be prohibitive. However, biological laboratories preparing these substances usually have on hand batches that are a little too toxic to meet the requirements of safety for the treatment of human beings and yet may prove quite acceptable for the treatment of the lower animals and obtainable at a much reduced price. For example, arsphenamin for administration to human subjects must be tolerated by rats in a dose of 0.120 gm. per kilo of weight by intravenous injection, but compounds tolerated in but 0.050 gm. per kilo would still be satisfactory for the treatment of horses since the maximum amount advised above is at least five or more times less than this dose per kilo of weight. Neoarsphenamin is even safer. For the treatment of human beings it must be tolerated by rats in dose of 0.240 gm. per kilo by intravenous injection, but for horses compounds having a maximum tolerated dose of but 0.150 gm. per kilo are safe, inasmuch as the dose advised above is at least ten times smaller than this amount per kilo of weight. Curasson[26] has reported success in the treatment of trypanosomiasis of the lower animals in Soudan with "galyl" (phospharsphenamin), but since both arsphenamin and neoarsphenamin possess a higher degree of trypanocidal activity it would appear that either of these is to be preferred to galyl.

(*c*) *Tryparsamid* in dose of about 0.040 gm. per kilo dissolved in sterile water. For a 1600-pound horse this would be 30 gm. dissolved in 150 c.c. of water for intravenous or intramuscular injection. Owing to the cheapness of this compound, and the efficacy of intramuscular injections it would appear possible to use it on a large scale.

(*d*) *Atoxyl* in dose of about 0.010 gm. per kilo. For a horse weighing 1600 pounds this would be about 8 gms. dissolved in 80 c.c. of water for intravenous, intramuscular or subcutaneous injection. The cheapness and ease

of administration of this substance also permits it to be used on a large scale in the treatment of selected animals of special value.

Possibly atoxyl has been used more frequently than any other arsenical compound in the treatment of dourine, surra, nagana disease, debab, etc., especially of horses. As a general rule, tartar emetic has been administered at the same time.

Horses sometimes develop well-marked degrees of intolerance for both drugs, and due caution must be exercised.

(*e*) *Tartar emetic* in dose of about 0.010 gm. per kilo. For a 1600-pound horse this would be approximately 1000 c.c. of a 1-per cent. solution in water by intravenous injection. As previously stated, tartar emetic requires a dilution of about 1 per cent. and intravenous injection. Subcutaneous and intramuscular injections produce severe local reactions. Sergent and Donatein[27] have recently reported upon the successful treatment with tartar emetic of trypanosomiasis of dromedaries ("debab"); Sergent and Foley[28] in a comparative study of "afridol" (a benzidin dye), trypan-blue and tartar emetic in experimental trypanosomiasis of dromedaries, observed best results after a combination therapy of atoxyl and tartar emetic. Van den Branden[29] has used with success tartar emetic, atoxyl and tryparosan in the treatment of surra (*Tr. evansi*) infections of cattle.

(*f*) *Sodium antimonyl thioglycollate* in dose of about 0.02 gm. per kilo. The compound may be given intravenously or *intramuscularly* in a 1 per cent. solution and for a 1600-pound horse the dose would be 150 c.c. of a sterile 1-per cent. solution. The particular advantage of this new compound is the fact that it may be administered intramuscularly without local irritation.

(*g*) At the present time "*Bayer* 205" is being extensively employed in the treatment of trypanosomiasis and favorable reports have been rendered by Baerman,[30] Migone and Osuna[31] and Miessner.[32] For horses the dose may be 2 to 4 gm. in 10 c.c. of saline solution injected intravenously every 8 days.

In the *treatment of dourine, surra, nagana disease, mal de caderas, debab* and other trypanosome diseases of horses, cattle, camels, etc., repeated doses are required, accompanied by blood examinations for parasites very much along the same lines previously outlined for the treatment of African trypanosomiasis of human beings.

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PART IV

CHEMOTHERAPY OF SPIROCHAETAL DISEASES OTHER THAN SYPHILIS

CHAPTER XXI

PRINCIPLES OF EXPERIMENTAL SPIROCHAETOSIS IN RELATION TO CHEMOTHERAPY AND THE TECHNIC OF SPIROCHAETICIDAL TESTS

Owing to the world wide distribution and tremendous economic importance of syphilis it is not surprising that the chemotherapy of experimental spirochetic infections has always held the first place of interest and importance. Furthermore, the occurrence of other spirochetic and leptospira diseases among human beings as relapsing fever, frambesia tropica, infectious jaundice, rat-bite fever, Vincent's angina, etc., have added to the importance of chemotherapeutic investigations in spirochetic diseases. Owing to the greater difficulties surrounding chemotherapeutic investigations in experimental syphilis and the longer time required for the evaluation of results of studies with any one compound or group of substances, less work has been done than in the chemotherapy of experimental trypanosomiasis, although, since the discovery of arsphenamin and neoarsphenamin, a very large number of new compounds have been brought forward for the treatment of syphilis. In many instances, however, these new compounds have not been subjected to thorough preliminary studies for toxicity and spirocheticidal activity in the lower animals but rushed too quickly into the treatment of human subjects.

Influence of Technic upon the Evaluation of the Results of Spirocheticidal Tests.—It cannot be too strongly emphasized that the values expressed in the following chapters are to be broadly viewed in their interpretation. It is not possible to place a biological test involving two living things (animal host and test parasite) upon a basis of mathematical accuracy. This is even more difficult in experimental spirochetic than in trypanosome infections because the lower animals display greater variations in natural resistance to infection, immunity develops more quickly, it is more difficult to numerically control the degree of infection and more difficult to find the spirochetes in the tissues of the infected animals. Just as in the chemotherapy of experimental trypanosomiasis, the animal employed, the number of infecting spirochetes inoculated, the interval elasping between the time of infection and the administration of treatment, are factors of importance influencing the results. Furthermore, it is to be borne in mind that *different investigators working with presumably the same substance may secure different results because of variations in the purity and exact chemical constitution of the compound employed respectively by each.*

Practical Value of Chemotherapeutic Studies in Experimental Spirochetosis.—Chemotherapeutic studies in experimental spirochetic infections of the lower animals produced by different organisms have not only greatly aided the advancement of our knowledge of the underlying or fundamental principles of chemotherapy, but have had a more direct bearing upon the treatment of disease of human beings than similar investigations in experi-

mental trypanosomiasis. The treatment of experimental relapsing fever of mice, for example, has completely revolutionized the treatment of relapsing fever of man since one or more administrations of arsphenamin cures the infection in both and was first discovered in experiments employing mice. The same is true of the treatment of experimental yaws or frambesia (*Sp. pertenuis*) infections of rabbits, which resulted in the discovery of the wonderful curative properties of arsphenamin and neoarsphenamin for this disease in both rabbit and man. Likewise the chemotherapy of experimental syphilis of the monkey and more especially of the rabbit, has had a direct and valuable bearing upon the treatment of human syphilis. In addition to these examples may be mentioned the application of knowledge secured in the chemotherapy of experimental spirochetosis to the treatment of rat-bite fever, Vincent's angina, bronchial spirochetosis, etc., of human beings.

Experimental Spirochete Infections Commonly Employed in Chemotherapeutic Investigations.—In Europe *Spirochæta recurrentis* (*Spirochæta obermeieri*), the cause of European relapsing or recurrent fever of human beings, has been mostly employed in experiments employing white mice and rats. *Spirochæta duttoni* and *Spirochæta kochi*, the causes of African relapsing fever, have been less frequently employed. In America *Spirochæta novyi*, the cause of American relapsing fever, has been used to some extent. All investigators have employed infected mice or rats and the four spirochetes produce almost identical symptoms and lesions in these animals, although *Spirochæta novyi* is regarded by Fraenkel,[1] Strong[2] and others as a distinct species, chiefly on account of the immunity phenomena. As a general rule light infections are not fatal and owing to the cyclic remissions, the experimental animals in chemotherapeutic studies must be kept under observation for many weeks (as long as 60 days according to Hata) before the results may be evaluated. It is possible, however, to infect mice so heavily that death occurs in a few days unless the parasites are destroyed by some medicament like arsphenamin or neoarsphenamin.

Spirochæta gallinarum, the cause of relapsing fever in fowls, has been employed by Ehrlich and Hata and apparently is more readily destroyed in experimental fowl infections than the spirochetes of relapsing fever of mammals; unfortunately, however, the rapid development of immunity in this infection with spontaneous recovery, reduces the value of this infection for studies in experimental chemotherapy.

Spirochæta pallida infections of rabbits is now commonly employed for experimental chemotherapeutic investigations in syphilis. The ease with which these animals may be infected and handled in large numbers, the frequency of skin, glandular, osseous and other lesions corresponding to those observed in human syphilis, render these experimental infections very useful.

Application and Utilization of Experimental Spirochetosis in the Development of Chemotherapy.—Comparative tests with various dye and arsenical preparations in the treatment of experimental trypanosomiasis (rat) and spirochetosis (*Sp. gallinarum* and *Sp. obermeieri*) have shown that the spirochetic infections are more readily influenced and cured. Mercurial compounds, which in sublethal amounts are without appreciable effect upon experimental trypanosomiasis in rats, are decidedly more efficacious in the treatment of rabbit syphilis and rats infected with *Sp. novyi* or *Sp. obermeieri*. Apparently these spirochetes are more susceptible to the destructive effects of mercurial compounds than are the various trypanosomes and in chemotherapeutic investigations with mercurial compounds experimental spirochetic infections are to be preferred. Substances without demonstrable

trypanocidal activity *in vivo* may be spirocheticidal; therefore, in chemotherapeutic investigations designed for the preparation of compounds for the treatment of syphilis or other spirochetic infections, the results of tests with trypanosome infections may be misleading and insufficient. On the other hand, compounds exerting trypanocidal effects are very apt to prove spirocheticidal; this is an established fact with arsphenamin, neoarsphenamin, sodium arsphenamin, sulpharsphenamin, etc., as discussed in Chapter XII.

Unfortunately, the low virulence of the spirochetes of relapsing fever for rats and mice, unless an overwhelmingly large infection is induced, the usual and frequent remissions and the tendency for the development of immunity and spontaneous recovery, reduce the value of these experimental infections for chemotherapeutic research. In fowl spirochetosis the rapid development of immunity and spontaneous recovery also interfere with the interpretation of experiments and introduce the error of ascribing curative properties to the agents under study which they do not actually possess.

Probably the treatment of syphilis of rabbits is best adapted for the development of experimental chemotherapy of syphilis. With arsenical compounds preliminary tests may be conducted with trypanosome infections, but some inorganic arsenical compounds may be without demonstrable trypanocidal activity in the maximum amounts that may be given rats and yet demonstrate some degree of spirocheticidal activity in rabbits. As a general rule, testicular infections are produced and the results of treatment evaluated by the rate of disappearance of spirochetes from the lesions, the clinical effects and lastly by inoculation tests for spirochetes in the lymph-glands. Insofar as the rate of disappearance of spirochetes from the testicular lesions is concerned, the results are available within a week after treatment is given, but the final results may require periods of observation as long as four months. For this reason, coupled with the greater expense of using and maintaining the large number of rabbits required for these tests and the lymph-gland control transplantations, rabbit syphilis tests can hardly be used for routine work, but reserved for the final testing of the more promising compounds developed for the treatment of human syphilis.

Much that has been stated in Chapter IX on the principles of experimental trypanosomiasis in relation to chemotherapeutic investigation is applicable to the present subject of spirochetic infections. One meets with similar variations in the virulence of the organisms and the influence of animal passage upon virulence and the results of therapeutic tests. The danger of "drug fastness" is just as important. As a general rule, the results of therapeutic experiments in spirochetic infections are more diffcult of proper interpretation and evaluation because of the relatively low virulence of the organisms for the lower animals and tendency for the development of immunity and spontaneous recovery.

Technic of Spirocheticidal Tests Employing Mice.—For these tests *Sp. recurrentis* (*Sp. obermeieri*) has been generally employed in Europe; the writer has employed *Sp. novyi.*

The strain is propagated in the usual manner by securing blood from a seed animal during a period when spirochetes are present in the blood and injecting fresh animals intraperitoneally. In order to avoid bacterial contamination, I have usually secured blood from the heart of the seed animal as described on page 207. With a sterile 1 c.c. syringe fitted with a gauge No. 22 needle and loaded with 0.5 c.c. of warm saline solution, about 0.1 c.c. of blood is readily aspirated from the heart and 0.2 c.c. of the mixture injected intraperitoneally into several mice. As a general rule, these transmissions are required only twice a month to maintain a strain of moderate virulence.

For conducting chemotherapeutic tests, adult mice of approximately 15 to 20 gm. weight are employed. These may be infected with relatively small numbers of organisms, in

which case the mice survive for several weeks or indefinitely with frequent remissions or they may be infected heavily in which case the majority of animals succumb during the first two weeks of the experiment with organisms almost constantly present in the blood as in trypanosomiasis. The latter method has proven the more useful in my work.

The blood of the seed animal is first examined by placing a drop from the tail on a slide, and examining microscopically. If large numbers of spirochetes are present, the animal is anesthesized and bled into about 5 c.c. of warm 1-per cent. sodium citrate in physiological saline solution, by severing the vessels of the neck and collecting the blood by means of a funnel. A drop of this mixture is then examined microscopically and diluted, if necessary, to show about 50 spirochetes in each field. Animals are now injected intraperitoneally with 0.2 c.c.; the injections are made as rapidly as possible because the citrate-saline solution is toxic and tends to destroy the spirochetes with the production of irregular infections in a series of mice. In every therapeutic test at least 4 infected mice are not treated, but kept for controls. Twenty-four hours later, the majority of mice will show a few organisms in the blood varying from 3 to 5 in each field. The drug is administered at this time either by intravenous or intraperitoneal injection.

Each animal is carefully weighed after fasting for at least 12 hours, and the dose given per gram of weight. Intravenous injections are given in a tail vein, the technic being described in Chapter II.

Daily microscopical examinations are made of the blood from the tail of each animal, including the controls, and the results recorded in the same manner as described on page 204 for the trypanocidal tests. In the untreated controls, the organisms rapidly proliferate in the blood so that by the second day they may reach 50 or more per field (Fig. 14), and on the third day appear in abundant masses. Nearly all untreated animals succumb during the crisis from the 5th to 12th days after inoculation.

FIG. 14.—BLOOD OF A RAT INJECTED WITH SPIROCHAETA RECURRENTIS.
Drawing made from a dark-field illumination just before the administration of arsphenamin.

In lighter infections, a relapse occurs on or about the fourth day, which lasts approximately three days followed by the presence of spirochetes in the blood for two or three days and another relapse. Third and fourth relapses may also occur, the periods of intermission becoming longer and tending to spontaneous cure. Great care must be exercised in making the microscopical examinations, because spirochetes may escape detection. As a general rule, the experiment can be terminated arbitrarily at the end of three weeks, although mice showing no spirochetes at this time are not necessarily sterile but may be in a period of remission of the infection. As shown by Breinl and Kinghorn,[13] inoculation of the blood in this apparent parasite-free period may yield positive results, and for this reason a negative microscopic examination of the blood cannot be accepted as evidence of sterility. Hata[4] has kept mice under observation for as long as 60 days before terminating an experiment and draws particular attention to errors due to individual variation in susceptibility of mice and the influence of acquired immunity. As a final test of cure, Hata sometimes re-inoculates the mice with larger numbers of spirochetes; in animals that had been promptly cured re-infection will occur whereas in uncured animals re-infection does not take place, owing to the acquired immunity engendered by surviving spirochetes even in long periods of remission during which the blood is apparently free of parasites by microscopical administration.

By comparing the results with the untreated controls, it is possible to express the results at any arbitrary period. I always include arsphenamin in each lot of tests, in order to compare new compounds with this substance and to enable an evaluation in terms of the "arsphenamin coefficient" as described in Chapter X.

Technic of Spirocheticidal Tests Employing Rats.—These tests are sometimes unsatisfactory, owing to the fact that rats are capable of rapidly developing an immunity to *Sp. obermeieri* and *Sp. novyi*, which may result in complete recovery soon after infection and lead to error in the interpretation and evaluation of results.

Young rats of about 100 gm. are to be preferred. When inoculated intraperitoneally with 0.5–1.0 c.c. of blood containing 30–50 spirochetes in each microscopic field, the spirochetes appear in the blood within 24 to 40 hours, unless the strain is highly virulent, in which case they may appear as early as eighteen hours after inoculation. As a general rule, the spirochetes remain the blood for about 3 to 4 days, when they gradually disappear. Relapses are rare and completely recovery with resistance to re-infection is the rule.

In my experiments, *Sp. novyi* has been employed, the rats being inoculated intraperitoneally as described and injected with the drug intravenously 24 hours later. In each experiment at least four untreated controls were included. Medicaments are given according to body weight and the tail blood of each animal examined microscopically each day for about four days. Under these conditions, the results are expressed according to the smallest amount of medicament per kilogram of body weight capable of sterilizing the animal 48 hours after treatment (72 hours after infection).

Experimental Syphilis of the Rabbit.—In 1906 Bertarelli[5] succeeded in transmitting *Spirochæta pallida* to rabbits by inoculation of the anterior chamber of the eye with the production of syphilitic keratitis. This discovery showed that these animals were susceptible and opened up a means for the experimental chemotherapy of syphilis. In 1907 Parodi[6] succeeded in inoculating the testicles of rabbits and this procedure is now commonly employed in preference to corneal inoculations. In 1908 Hoffman, Löhe and Mulzer[7] succeeded in producing infection of the skin of the scrotum of rabbits, and Levaditi and Yamanouchi[8] succeeded in inoculating the prepuce. Since then the subject of experimental rabbit syphilis has been extensively studied and especially in America by Brown and Pearce,[9] who have brought this animal infection to a stage of development quite near that of the human disease.

Choice of Organism.—From the standpoint of experimental infections for chemotherapeutic investigations, preference should be given a strain of spirochetes that has been passed from rabbit to rabbit for many generations. In this manner the strain becomes adapted to the tissues of this animal, probably acquires or at least maintains a high degree of virulence and produces uniform lesions so essential for the proper interpretation and evaluation of chemotherapeutic studies. Brown and Pearce, in their extensive and valuable studies over a period of four years, employed two strains of *Spirochæta pallida*, one having been isolated by Zinsser and Hopkins in November, 1913, from a mucous patch (Strain A) and the second by Nichols from the spinal fluid of a case of neurorecidive in June, 1912. My own work has been largely conducted with the latter strain.

Choice of Animals.—Young animals with well-developed testicles and thin scrotums are to be preferred to old animals with redundant, thick and fibrous scrotums and atrophic testicles. The small or medium-sized albino, common gray, brown or Dutch belt rabbits are quite satisfactory for the production of testicular lesions and the general lesions of syphilis. As recently shown by Chesney[9a] young rabbits react to an intratesticular inoculation of the syphilitic virus with a slightly more marked initial lesion than do older animals similarly inoculated, and tend to postpone slightly the appearance of subsequent generalized lesions, which are less severe in character and extent.

Methods for Propagating Strains of Spirochæta Pallida.—Practically the only reliable and serviceable method for propagating *Spirochæta pallida* is from rabbit to rabbit; cultures in artificial media soon lose their virulence. At suitable intervals stock rabbits may be infected by scrotal or testicular inoculation with material from the testicles of syphilitic rabbits, but lymph-gland emulsions are especially to be recommended.

As shown by Brown and Pearce,[10] spirochetes survive in the lymphoid tissues for long periods of time even during latency in which there are no clinical evidences of infection.

For this reason, Pearce and Brown have advocated propagation of the strains employed for chemotherapeutic work by injecting fresh animals with emulsions of the ground up glands of seed syphilitic rabbits. Under this plan it is merely necessary to keep a sufficient number of infected animals to guard against loss of the strain by their death. Serial transfers may be dispensed with.

When it is desired to recover the organism, a popliteal node may be excised under anesthesia and with aseptic precautions, minced, and ground in a mortar; an emulsion is then prepared by the addition of about 1.5 c.c. of sterile normal saline solution with further grinding. It is not necessary or advisable to use foreign substances, as sand, to aid in the grinding of the material. The resulting fluid is simply aspirated into a small syringe fitted with gauge 22 needle and about 0.5 c.c. injected into a testicle of one or more normal rabbits. In order to allow ample time for the development of a testicular infection, the inoculation should be made 6 to 8 weeks before the organism is needed for the infection of a larger number of animals for chemotherapeutic or other purposes.

Choice of Lesion.—As previously stated inoculation of the cornea is no longer employed. For purposes of chemotherapeutic investigations testicular lesions are more satisfactory and the percentage of "takes" with a good laboratory strain is very high; but they are on the whole of less value as objects of clinical study than skin (scrotal) inoculations, because a large part of the process takes place in parts hidden from direct observation and the infection is of a less stable character. Skin inoculations, on the other hand, are more akin to the mode of infection in the human being and the lesions are more analogous to the primary lesions of man. The lesions are likewise more accessible to observation and the results of chemotherapy readily observed.

However, the testicular lesions are just as easily examined for spirochetes by dark-field examination and the results of treatment may be evaluated with comparative ease by noting the influence upon the size of the lesion and disappearance of the organisms. Chesney has tried intradermal inoculations but observed that generalized syphilis is less prone to develop than in the case of intratesticular inoculations. However, even though the primary reaction to intradermal inoculation may be slight or absent, general invasion of the body with spirochetes may take place, as evidenced by positive lymph gland transfers, even though no generalized lesions may be observable. Chesney and Kemp[10a] have also shown that rabbits can be successfully infected with syphilis by the inoculation of surface wounds with virulent strains of *spirochæta pallida.*

Technic and Course of Testicular Syphilis of the Rabbit.—A seed animal should be chosen presenting a well-developed testicular lesion and showing numerous actively motile spirochetes by dark-field examination. *But care must be exercised against using testicular lesions at the time of regression and showing large numbers of agglutinated immobile spirochetes. The infectivity of these is greatly reduced and approximately* 10 *times more are required for successful inoculation.* In this connection it is interesting to record in passing that Miss Rule and I have kept excised syphilitic testicles from rabbits in a refrigerator at about 12°C. for as long as two weeks, finding that the spirochetes were still slightly motile at end of this time and capable of infecting rabbits upon testicular inoculation. I mention the matter here to indicate that the organisms may survive for long periods of time under such conditions and that undue haste in transplanting is not required.

The material or a portion of it is placed in a sterile mortar and finely minced with sterile scissors, after which enough sterile saline solution is added to moisten the entire mass. The material is then rubbed to a paste, more salt solution being added so as to give about two or three spirochetes in each microscopic field by dark-field examination. Usually two to five volumes of salt solution are required to one of tissue. The fluid emulsion is then aspirated into a small sterile Record or Luer syringe fitted with a sharp, smooth guage 22 needle about $\frac{5}{8}$ inch in length. The scrotum of the animal to be inoculated is sponged with 1-per cent. tincture of iodin and 0.2 to 0.5 c.c. of the emulsion injected into the center of each testicle (Fig. 15). As shown by Chesney and Kemp[10b] the size of the dose of the infecting material exerts an appreciable effect upon the duration of the incubation period in experimental syphilis of the rabbit. Other things being equal, the larger the dose the shorter is the incubation period. However,

the size of the inoculum was not found to exercise any marked effect upon the course of the disease as a whole; rabbits inoculated with minimal amounts may run a malignant course.

In from 7 to 10 days, spirochetes may be found in the testicles by dark-field illumination; likewise histological changes are usually present, although

FIG. 15.—TECHNIC OF TESTICULAR INOCULATION OF THE RABBIT.

the testicle may appear normal and show no gross signs of infection. In from 3 to 4 weeks after inoculation, however, clinical signs of infection are usually

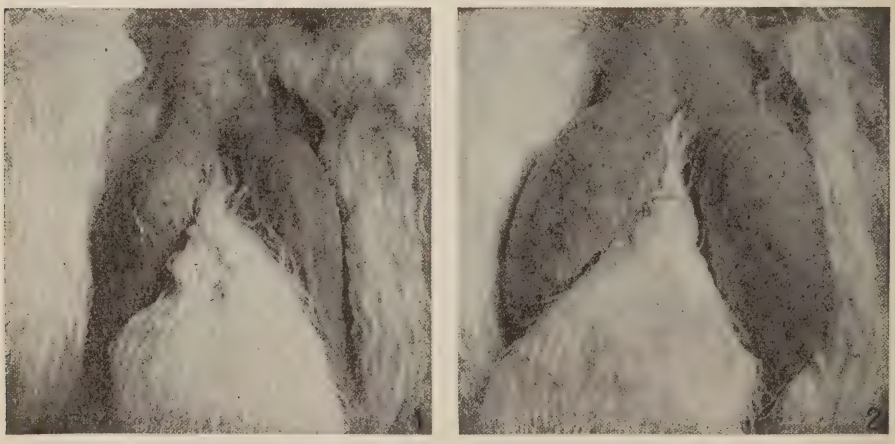

FIG. 16.—ACUTE SYPHILITIC ORCHITIS IN A RABBIT.
Normal testicles on the left; well advanced, acute syphilitic orchitis with beginning congestion and edema of the scrotum on the right. (Brown and Pearce.)

apparent, unless the inoculation has been from old, inactive, or regressing lesions in which case the period of incubation is of longer duration.

As a general rule, the testicle first presents either a diffuse swelling with increased tension or a small circumscribed focus of induration situated at the point of inoculation. The testicle may now become acutely swollen with reddened skin and considerable edema (Fig. 16), terminating in a crisis followed by a slight secondary cycle of proliferative reaction. This type of reaction is especially likely to develop when rabbits are inoculated at short intervals from acute progressive lesions. Dark-field examination shows that the spirochetes increase with the orchitis, so that by the time the lesions have reached their height, actively motile organisms are present in large numbers. At the crisis, the spirochetes suddenly lose their motility, collect in conglomerate masses, rapidly diminish in numbers and show loss of motility and evidences of degeneration. After passing through a crisis of this kind, they may again increase in numbers and motility presaging a renewed activity on the part of the orchitis, these parallel cyclic changes continuing throughout the existence of the local lesion.

On the other hand, enlargement of the testicle may take place more slowly and steadily for 2 or 3 weeks by which time the organ presents a large, smooth or slightly nodular lesion of extreme hardness. Instead of a pronounced crisis, this lesion regresses more slowly, the testicle becoming soft and gradually reduced in size leaving a diffuse residual induration. This is followed by cycles of activity and remissions for a considerable period of time. This type of lesion is especially apt to develop when inoculations are made at long intervals or from chronic indurative lesions. Dark-field examination reveals spirochetes, but not usually in as large numbers as met with in the acute exudative lesions.

In addition to these two typical forms of orchitis, one sometimes sees lesions partaking of the features of both, what may be designated as subacute orchitis in which the features of acute exudation and infiltration are associated with proliferative changes in the tissue cells. In practically all instances, the infection ultimately involves the entire organ—a panorchitis—with lesions in the parenchyma, tunics, epididymis and cord, and in many instances the skin as well.

During the latter part of the orchitis the spirochetes become fewer and more difficult to find. Sometimes spirochetes are found in one portion of the testicle and not in another, but as a general rule the active areas and quiescent lesions, not regressing, show large numbers of motile spirochetes.

The duration of these local lesions is quite variable, being inversely proportional to their intensity. In some animals the entire reaction is one intense cycle of acute reaction terminating in 4 to 6 weeks after inoculation. In others, the infection continues in successive cycles, but the period of active infection rarely lasts longer than 2 to 4 months. Inactive or latent lesions and residual lesions in the epididymis, skin, and tunics sometimes persist for from 4 to 12 months.

Technic and Course of Scrotal Syphilis (Chancres) of the Rabbit.—Young animals should be chosen in which the scrotum is thin and as free from hair as possible. Large and old rabbits with more or less ample or redundant scrotums are poor subjects for the production of scrotal chancres. The best animals are those just approaching full maturity. Belgian and Flemish giants give poor results, while the small albinos, grays, browns and Dutch belts are more satisfactory.

The technic of inoculation commonly employed is essentially the same as that described by Tomasczewski,[11] which consists in the implantation of a small piece of infected tissue in a pouch prepared for it in the scrotum.

The tissue is generally secured from the testicle of a seed animal as described above, being removed with as little contamination as possible and cut into pieces measuring from 2 to 5 mm. in diameter. Due to unequal distribution of spirochetes in infected tissues as well as to inequalities in virulence, inoculations by this method may not be uniform. But, according to Brown and Pearce, these difficulties may be overcome by the observance of certain precautions: First, the use of infected tissues at a relatively early stage of the infection or only at a period when the infection is actively progressing; second, the use of only such portions of a lesion as are actively growing at the time; and third, as a means of insuring an adequate dose of organisms when any doubt exists, the use of pieces of tissue as large as can conveniently be used without causing necrosis and sloughing of the surrounding tissues.

The animal to be inoculated is fastened to an operating board or held by an assistant and the scrotum sponged with 1-per cent. tincture of iodin, stronger antiseptics sometimes destroying the spirochetes and irritating the skin. Anesthesia is not required. The superficial layers of the skin are snipped with scissors and a small pocket made with forceps in the subcutaneous tissue as superficially as possible, in which a fragment of infected tissue is introduced for the distance of at least half an inch. Or the inoculation may be made with a trocar needle of 8 to 9 guage. Brown and Pearce have also produced superficial lesions by injecting intracutaneously or subcutaneously 0.1 to 0.2 c.c. of a saline suspension of testicular material containing at least 5 to 10 spirochetes in each dark-field and prepared as described above. This method has the advantage of causing less trauma but sometimes results in producing a more diffuse lesion than desired.

With the *method of tissue transplantation* the period of incubation varies from 10 days to 2 weeks, but may be prolonged for months in cases of inoculation of few organisms or of organisms of low vitality. The early reaction is a combination of irritation by the inoculated tissue, wound healing and the specific reaction. The traumatic reaction usually subsides in 2 to 3 days and healing of the incision is generally complete within 7 to 10 days, although sometimes an ulcer develops over the transplanted tissue and especially if bacterial infection has occurred.

Edema and congestion of the scrotum now develops with necrosis of the skin over the transplants and the formation of hard dry crusts which show a line of demarcation at their periphery. The edema and congestion gradually involve the scrotum of both testicles due to the inherent tendency of *pallida* infections to spread beyond the local confines of the initial lesion; multiple chancres may develop.

With the *method of injection of spirochete emulsion*, the incubation period may be as short as 1 week or delayed for 4 weeks; usually lesions developed within 10 days to 2 weeks after inoculation.

Usually the first sign of the chancre is a small, soft, gelatinous swelling of a rose-pink color situated in the papillary and reticular layers of the skin and frequently associated with a rich vascular network or the lesion may first appear as a more superficial minute translucent nodule or as an opaque, white nodule of extreme hardness in the depths of the skin and usually connected with the outer surface of the tunica vaginalis. From these initial points the lesions develop into elevated masses with necrotic centers covered with dry crusts and gradually involving the whole scrotum, the cause and development of the lesions varying somewhat according to the character and location of the initial lesion.

The resulting chancres from either method of inoculation are subject to wide variation in extension and appearance. Usually they are nodular and

of irregular spherical form, with small sharply circumscribed areas of superficial ulceration and accompanied by extreme induration. Or they may appear as lenticular and discord lesions of a more flattened character, with more widespread necrosis and a lesser degree of induration. Sometimes the lesions are very atypical and diffuse with marked fibrosis of the scrotum, running a prolonged course and sometimes with the development of small areas of necrosis.

The subsequent course of these chancres is very irregular. As a rule, a period of growth lasts for 2 to 3 weeks followed by an interval of inactivity. As long as the lesion is active or quiescent it retains its appearance unaltered, but as soon as regression sets in the skin becomes lax and wrinkled, the induration less and the lesion smaller in size. As a general rule, three or four cycles of activity and regression will occur, but not infrequently is limited to a single cycle; the duration of the scrotal lesion may vary, therefore, from 1 to 12 months or longer. In the majority of instances the infection terminates by degrees, healing taking place with the production of a scar and requiring about 4 to 6 weeks for completion.

Spirochetes are to be found in the secretion from these lesions, but their number is subject to wide fluctuation and bears no constant relation to the size of the lesion since large numbers may be found in relatively small and insignificant chancres. As is true of testicular lesions, most spirochetes are to be found in the developing progressive lesions and especially at the periphery of chancres of cellular or mucoid character. In regressing healing and fibrous lesions they are apt to be scarce and one may fail to find them in dark-field examinations. These facts should be kept in mind in the course of chemotherapeutic investigations in order to avoid the error of ascribing spirocheticidal properties to a drug under study when the failure to find spirochetes may be due to the character of the lesion.

Furthermore, the size of the chancre is no index to the degree of general infection that may follow; some of the most severe generalized infections may follow the most insignificant chancres and indeed, large chancres tend to inhibit the development of other focal infections, as discussed later in more detail.

Generalized Syphilis of the Rabbit.—In addition to the tendency for local dissemination of syphilis in the scrotum and testicles of rabbits, described in the preceding sections, it is now well established that a more widespread distribution may occur in which distant tissues and organs may be involved. In 1909 Uhlenhuth and Mulzur[12] reported metastatic infection from one testicle to the other testicle and scrotum and a second case of metastasis to an uninoculated testicle after removal of the infected testicle. At about the same time Ossola[13] and Truffi [14] demonstrated virulent spirochetes in the inguinal lymph-glands of infected rabbits and since then metastases of this kind have been frequently noted and especially studied by Brown and Pearce, the latter investigators showing that the rabbit may develop practically all the lesions of syphilis known to occur in syphilitic human subjects like syphilitic adenitis, cutaneous and muco-cutaneous lesions, lesions of the eyes, bones, cartilage, tendons, etc. For a description of these and the literature upon the subject, I refer the reader to the excellent series of papers by Brown and Pearce.[15] Doubtless many of the lesions developed in rabbits have hitherto been disregarded or overlooked and ascribed to the effects of trauma or other infections until shown to be generalized metastatic lesions of syphilis by their extensive and thorough studies.

As shown by Brown and Pearce, when rabbits are inoculated in both testicles generalized lesions develop in about 20 per cent. of animals; when

inoculated in one testicle generalized lesions develop in about 50 per cent. of animals. This difference is ascribed to the production of immunity principles by the tissues of the testicles, tending to inhibit the dissemination of the spirochetes; the immunity is less when one testicle is infected than when both organs are inoculated.

Furthermore, if rabbits are castrated soon after inoculation generalized syphilis is especially apt to occur. For example, as shown by Brown and Pearce, if rabbits are inoculated in one or both testicles and castrated 2 to 14 days later, generalized syphilis occurs in practically all animals. Brown and Pearce have also shown that, if the testicular lesions are suppressed by the injection of a therapeutic agent (6 mg. per kilo of arsenophenylglycyl dichloro-m-aminophenol 14 days after testicular inoculation in their experiments), generalized syphilis may be produced in the majority of animals. These experiments show that in the rabbit the local lesion tends to inhibit the development of lesions elsewhere and that the reduction or supression of the local reaction by the use of any means that does not remove or destroy all of the spirochetes, removes this control and tends to increase the occurrence of generalized lesions and the severity of the infection.

For the production of generalized syphilis for purposes of chemotherapeutic investigations, one may inoculate rabbits in one or both testicles and castrate about 7 days later.

Of further interest in this connection are the observations of Brown and Pearce[16] that, by various experimental procedures, it was possible to produce different types of generalized infection with a definite sequence of tissue involvement or of tissue reactions, which made it possible to produce infection or to confer protection on a given group of tissues according to the nature of the means employed. These investigators have concluded that a wide variation in the clinical types of syphilis in the rabbit might be traceable to this group of experimental causes. This, of course, does not eliminate the possibility that there are different strains of *Spirochæta pallida* producing certain lesions by selective tissue tropism as first suggested by Noguchi[17] and later studied by Nichols,[18] Reasoner,[19] Levaditi and Marie,[20] Marie, Levaditi and Banu[21] and others discussed at length in Chapter XXXVI, but the experiments of Brown and Pearce demonstrate that great care should be exercised in drawing conclusions in this subject, since the distribution of spirochetes is apparently influenced to some extent at least by experimental factors aside from the possibility of some strains being endowed with the property of localizing specifically in certain tissues.

Criteria of Cure of Rabbit Syphilis.—In view of the fact that spirochetes may be found in the popliteal, inguinal and other lymphatic glands of syphilitic rabbits many weeks and months after inoculation and apparent recovery, it is doubtful if this animal is able to bring about complete cure unaided by antispirocheticidal drugs as is also apparently true of human beings. As stated in the preceding pages, syphilis in the rabbit tends to pursue a cyclic course of exacerbation and regression of lesions and the periods of latency may be so prolonged and the animal to all appearances so completely recovered and well, that one may readily enough fall into the error of regarding the animal as cured. If, however, the lymph glands are removed, ground up and the emulsion injected into the testicles of normal rabbits, the presence of living spirochetes may be demonstrated; for this reason *no rabbit can be regarded as having completely recovered from syphilis in chemotherapeutic investigations unless this lymph-gland test proves negative.*

As a general rule, rabbits are selected for chemotherapeutic experiments when the lesions are active and spirochetes are to be found by dark-field

examination. After the administration of the medicament, the results may be evaluated by noting and recording the following data: (*a*) The time when spirochetes have disappeared by thorough and repeated dark-field examinations of different lesions and different areas of the same lesion; (*b*) the clinical regression of the lesion or lesions; (*c*) the presence or absence of exacerbations of the old lesions and development of new lesions over a period of at least 2 months; (*d*) finally, the results of inoculation of an emulsion of lymph-glands into a fresh animal and its observation for a period of at least 2 months for the development of syphilis.

The complement-fixation test as a criterion of cure in rabbit syphilis is not reliable. In the first place, the serums of a large percentage of normal rabbits give positive Wassermann reactions as first discovered by Schilling and Hoesslin[23] and since amply confirmed by Casselman, Trist and myself[22] and others. By the use of a quantitative test employing graded amounts of serum, it can be shown that the serums of syphilitic rabbits acquire an increased power for complement fixation with tissue extracts, but I have frequently observed negative reactions with the serums of rabbits in the latent stages of syphilis when lymph-gland inoculation into fresh animals has disclosed the presence of living spirochetes.

Venereal Spirochetosis of Rabbits.—Apparently this condition of normal stock rabbits may be mistaken for the syphilitic lesions of the penis and anus. Ross and Bayon[24] in England, were the first to call attention to this condition associated with a spiral organism morphologically indistinguishable from *Spirochæta pallida* of human origin. Soon afterward, Artz and Kerl,[25] Jacobsthal,[26] Schereschewsky[27] and Klarenbeek[28] published observations of a similar nature. Brown and Pearce[29] state that they have never encountered the infection. Noguchi,[30] however, has recently encountered the condition in nine female and two male rabbits in the so-called normal stock of the Rockefeller Institute and has described the disease as follows:

"The lesions are situated on the vulva or prepuce and appear as scaly, papular, somewhat congested spots or patches, slightly elevated from the surrounding muco-cutaneous surface; they are not indurated to any marked extent as are usually the lesions produced by *Treponema pallidum* in similar regions. There is at first no exudate, but mechanical irritation of any kind induces a hyperemic condition of the lesions, and the dry scales fall off, leaving an exposed, granular surface. The exudate dries rapidly and forms a shining crust, dark gray or yellowish, removal of which is followed again by exudation, appearance of minute ecchymoses, and crust formation. In chronic cases the lesions may cover the entire genital region and extend to the anus.

"The minute initial papules, as well as the advanced lesions with crust, contain very large numbers of organisms perhaps slightly coarser and longer than the average type of *Treponema pallidum*. The length varies from 10 to 18 microns, the majority being from 10 to 12 microns for the average *Treponema pallidum*. Individuals as long as 30 microns have been observed (specimens of *Treponema pallidum* as long as 24 microns have occasionally been encountered in experimentally infected rabbits). The width is about 0.25 micron, spiral amplitude from 1 to 1.2 microns, spiral depth from 0.6 to 1 micron; the spirals, from eight to twelve in number are regular and deep. Rapid rotation and occasional bending at the middle are observed, but no vibratory or lashing movements. The organism belongs to the genus *Treponema*, and may be designated as *Treponema cuniculi*."

Noguchi attempted to transmit the disease by mating infected animals with normal animals and was successful in one instance. Schereschewsky,[31] however, was able to transmit the disease sexually with great ease, and utilized the method of transmission for testing the prophylactic value of different ointments.

Nichols and Walker[32] have likewise failed to find evidences of this disease among normal rabbits, but verified Noguchi's observations with a female animal furnished by Dr. Noguchi. According to Nichols and Walker, how-

ever, "natural spirochetosis of rabbits need not be a serious complicating factor in work on syphilis in rabbits, for the following reasons: (*a*) In natural spirochetosis the lesions occur on the penis and not on the scrotum. Gland transfers are negative. (*b*) A scrotal lesion can be produced by inoculation, but it can be distinguished from that of *Treponema pallidum* infection by its course. (*c*) In studies of generalized syphilis supposed to involve the genitalia, and in sexual transmission experiments, *Treponema cuniculi* may be a serious complicating factor."

More recently Warthin, Buffington and Wanstrom[33] have reported a thorough series of studies in eighteen cases of rabbit spirochetosis and regard cuniculi spirochetosis as "a local disease of epithelial surfaces, the clinical course and local and general pathology of which do not in any way resemble those of syphilis. It should not be called "rabbit syphilis," and the term "paralues cuniculi" should also be discarded. The spontaneous rabbit disease can be easily differentiated from pallida infections by its morphology, as shown in silver-agar cover-glass smears, and by the pathology of the lesions."

I have never seen the disease among rabbits in Philadelphia and very much doubt that investigations in experimental rabbit syphilis in the United States at least has been appreciably influenced or compromised by this infection. Apparently the disease is more common in Europe. At any rate it is necessary to choose animals with care for experimental inoculation with *Sp. pallida* or *Sp. pertenuis*, and to carefully study and reject any that show infections about the genitalia and anus.

Experimental Frambesia (Yaws) of the Rabbit.—Shortly after the discovery of *Spirochaeta pallida* Castellani discovered *Spirochaeta pertenuis* as the cause of frambesia, a disease of tropical countries variously designated as "yaws," "pian," "coko," etc. The similarity between the two spirochetes is so close that differentiation is only possible by cross immunity tests.

In 1907, Neisser, Baermann and Halberstadter,[34] succeeded in transmitting the disease to the higher apes in Sumatra and demonstrated that it was a distinct entity rather than a tropical form of syphilis. In 1909, Nichols,[35] succeeded in infecting the eyebrow of a monkey (*Macacus rhesus*) by rubbing an abrasion with a piece of yaw nodule removed from a human subject. Nichols then succeeded in inoculating the testicles of rabbits with material from the monkey and thereby opened up a means for further immunological and chemotherapeutic studies in frambesia. A little later, Nichols[36] also succeeded in producing scrotal lesions (chancres) with *spirochaeta pertenuis* and since then other investigations have confirmed his observations. Both Nichols and Schamberg and Klauder[37] have failed to infect rabbits by inoculation of the anterior chamber of the eye. According to Nichols, large white rabbits are to be preferred to small and immature animals; gray, brown and black animals are less likely to become infected.

The testicles are inoculated by injecting an emulsion of yaw material containing large numbers of actively motile spirochetes obtained either from a human case of yaws or an infected rabbit, in exactly the same manner as described for the production of rabbit syphilis; or a small bit of the yaw tissue may be planted into the substance of the testicle or just beneath the scrotal skin, but injections of the emulsion directly into the substance of the testicle yields a higher percentage of successful inoculations.

Both Nichols and Schamberg and Klauder observed that only 50 per cent. of rabbits are successfully inoculated direct with human material; it is well therefore, to always inoculate at least 6 rabbits for the primary "takes" in order to insure 2 or 3 successful inoculations. Later a higher percentage of "takes" are secured by passage of the spirochete from rabbit to rabbit as in the propogation of *Spirochæta pallida*.

Direct inoculation of the rabbit testicle with human material is followed by a primary incubation of about 5 weeks; after several transfers in rabbits this period is shortened to 3–4 weeks, and even to 2 weeks as reported by Nichols. Lesions of the scrotum may develope in about 23 days, which is a much shorter incubation than in syphilitic chancers.

Both the testicular and scrotal lesions are almost indistinguishable from the lesions of syphilis (Fig. 17). Nodules develop varying in size from a pea to that of an olive, with necrosis of the tubules, infiltration of small lymphocytes and the production of new fibrous tissue (Nichols).

The organs are enlarged and edematous; a palpable node is present in each organ. A dark field examination of the serum obtained on puncturing each node disclosed many spirochetes.

It is known that in human frambesia the spirochetes may be found in the spleen, lymphatic glands, bone-marrow and sometimes in the blood. The distribution however, is seldom as extensive as occurs in syphilis, the cardio-vascular system for example, commonly escapes. The brain and spinal cord are likewise less liable to invasion with *Spirochæta pertenuis*. In yaws therefore, the spirochetes are more apt to invade the skin, mucous membrane of the nose and pharynx, the joints and periostium; Pearce and Brown[38] have recently described the various lesions with considerable detail.

Aside from the successful inoculation of monkeys and rabbits, yaws has not been transmitted to other animals as mice, rats and guinea-pigs.

FIG. 17.—TESTICULAR LESIONS OF RABBITS CAUSED BY SPIROCHAETA PERTENUIS. (Schamberg and Klauder.)

Methods for Determining the Spirocheticidal Activity of Substances in Vitro.—As stated in Chapter II, compounds are sometimes prepared in the course of chemotherapeutic studies which possess such high toxicity when administered to living animals, that sufficient amounts cannot be administered to appreciably influence a spirochetic infection. Under these conditions it is sometimes necessary to determine the spirocheticidal activity of a medicament *in vitro* or by a combined *in vitro in vivo* test.

Technic of Microscopical Methods.—With *Sp. novyi* (American relapsing fever) I have successfully employed a method exactly similar to that described in Chapter X for direct microscopical tests with trypanosomes. The blood of an infected mouse is employed and the highest dilution of the medicament is determined which produces complete loss of motility of the spirochetes. As is true of the trypanosome tests by this technic, the results are only approximately correct, because loss of motility of the spirochetes does not necessarily indicate their complete destruction.

With Spirochæta pallida direct microscopical tests may be conducted employing pure cultures in a field medium or emulsions of testicular tissue of rabbits showing sufficient numbers of spirochetes. The latter is prepared by removing a syphilitic testicle at a time when dark-field examination shows the presence of large numbers of actively motile organisms. The tissue is cut into small pieces and throughly rubbed up in a mortar with a few cubic centimeters of warm saline solution. This emulsion is now briefly centrifuged to remove the tissue fragments and the supernatant fluid employed. The culture or tissue preparation should contain such numbers of spirochetes that each microscopic dark field shows at least 20 to 30 organisms.

Dilutions of the medicament are prepared in test-tubes or vials and one small loopful (3 mm. loop) of each placed in the center of a thin cover-glass. To each is now added a loopful of the spirochete suspension followed by thorough mixing. The cover-glass is then placed on a thin microscopical slide and the material evenly spread. It is necessary to work with small amounts of material in order not to float the cover-glass with the production of cross currents when each is examined by dark-field illumination. Generally three cover-glasses can be placed on one slide. Each may be ringed with a mixture of vaselin and paraffin and kept at room temperature or at 37°C. in a small oven. Controls are

always included in which a loopful of the spirochete suspension is mixed with a loopful of saline solution.

Each preparation is examined by dark-field illumination at the end of 5, 10, 15 and 30 minutes; longer periods are sometimes employed providing the controls show the presence of motile spirochetes. The results are evaluated according to the highest dilution of medicament causing loss of motility of the majority or all of the spirochetes in a given period of time. These results do not, of course, necessarily indicate the complete destruction of the organisms.

Technic of Combined in Vitro in Vivo Method.—This test is conducted in exactly the same manner as the trypanosome test described on page 216. In working with *Sp. recurrentis*, the blood of a mouse is employed or a mixture of the bloods of several mice showing large numbers of actively motile apirochetes. Care should be exercised against using the blood of an animal in which the spirochetes are agglutinated and feebly motile or quiescent, which is generally observed just before or during a crisis of the infection. Mixtures of varying dilutions of the medicament and the citrated spirochete blood (0.5 c.c. of each) are made in small sterile test-tubes as previously described and kept in a water-bath. Controls are always included of mixtures of citrated blood (0.5 c.c.) and saline solution (0.5 c.c.). Since saline solution is toxic for these spirochetes the period of exposure should not be longer than 30 minutes when 0.5 c.c. of each mixture is injected into mice intraperitoneally and the animals observed for infection with daily examinations of the tail blood. As a general rule, tests of this kind employing *Sp. novyi* have not yielded as regular or clear-cut results as have been observed with *Tr. equiperdum* and *Tr. brucei*.

An exactly similar test has been employed with *Spirochæta pallida*. An emulsion of rabbit syphilitic testicle is prepared in saline solution and briefly centrifuged. The supernatant suspension of spirochetes should show at least 20 organisms per dark field.

Varying dilutions of the medicament in amount of 0.5 c.c. each are mixed in small sterile test-tubes with 0.5 c.c. of spirochete suspension and the mixtures kept in a water-bath for a given period of time, when 0.5 c.c. of each mixture is injected into the testicles of rabbits and the animals observed for the development of syphilis. Experiments have shown that under these conditions the spirochetes remain alive and infective in the controls (0.5 c.c. of saline solution and 0.5 c.c. of spirochete suspension) for several hours.

One drawback to the method is that some compounds in a spirocheticidal dilution are capable of exciting inflammatory changes in the testicle. Furthermore, the action of a medicament may be continued after the mixtures are injected, as previously stated in connection with the trypanosome test by this method; for this reason I have designated the test as a combined *in vitro in vivo test*.

Technic of in Vitro Culture Method.—With the spirochetes that have been successfully isolated and cultivated *in vitro* as *Spirochæta pallida, Spirocheta refringens, Spirocheta microdentium*, etc., it is possible to conduct *in vitro* tests after the technic employed by Akatsu and Noguchi.[39] These tests may be of a spirochetostatic or spirocheticidal character.

Technic of Dark-field Examination for Spirochetes.—While the spirochetes of human and fowl relapsing fever may be readily found by simple microscopic examination of the blood, *Spirochæta pallida*, because of its low refractive index, is seen when alive with difficulty by the ordinary microscope. By far the most successful and easily applied method of finding the organism in syphilitic lesions of the rabbit and human subject, is by the use of the dark-field method. The technic of this examination is as follows:

1. The apparatus required is: (*a*) *A powerful light* with lens to focus the rays on the mirror of the microscope. The usual arc light shown in Fig. 18 is excellent; a drawback is that it requires frequent adjustment of the carbons unless an automatic lamp is used. Outfits equipped with madza lamps are also available and possess the advantage of furnishing a steady light with less heat. (*b*) *A funnel stop* for the ordinary $\frac{1}{12}$th oil-immersion lens to reduce the light and (*c*) *A dark-field condenser*. This condenser may be purchased fitted to be screwed into the substage after removal of the usual Abbé condenser or in the form of a slide to be clamped to the stage of the microscope over the ordinary condenser. When much dark-field work is required it is advisable to provide a microscope fitted with the special substage condenser to be used alone for these examinations.

2. Thin or medium slides should be used; thick slides do not permit focussing of the dark field. The slides should be perfectly clean and preferably free of scratches. Thin cover-glasses are also required.

3. In the examination of a chancre (human or rabbit), the lesion should be superficially cleansed, in order to remove the many contaminating organisms on the surface. Most of the surface crust should be removed and a little serum squeezed out from the deeper tissues.

A drop or loopful of this material may be placed on the slide; it is sometimes advantageous to mix it with a drop of distilled water, but *care should be exercised not to use too much material or diluent in order to prevent floating of the cover glass* and the setting up of cross

FIG. 18.—OUTFIT FOR DARK-FIELD EXAMINATION FOR SPIROCHETES.

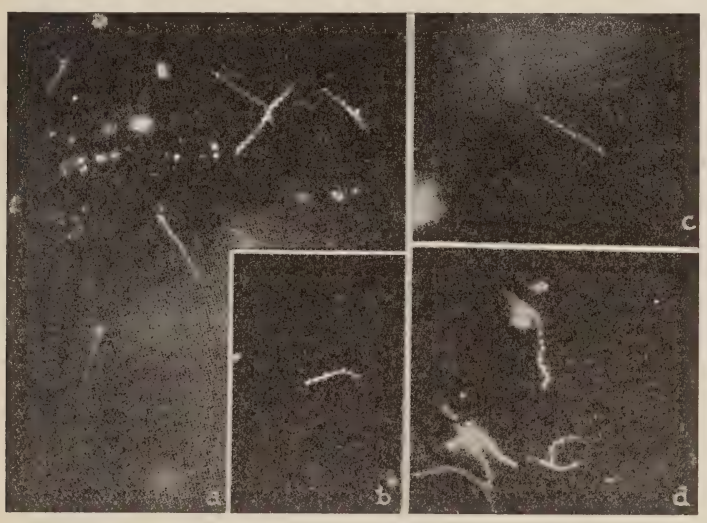

FIG. 19.—SPIROCHAETA PALLIDA.

Dark-field views; magnification × 1000. (a) From experimental syphilitic orchitis in the rabbit. (b and c) From human chancre. (d) From a pure culture. (Noguchi.)

currents which prevent proper examination. For the examination of testicular lesions in rabbits or enlarged lymph-glands in rabbits or human subjects, material may be secured by puncturing with a sterile No. 26 or No. 22 needle fitted to a 1 c.c. syringe and aspirating a sufficient amount of secretion for this examination.

4. A drop of immersion oil is placed on the under surface of the slide (this is preferable to putting a drop of oil on the condenser), and another drop on the cover-glass. The slide is then gently placed on the condenser so that the bottom drop of oil spreads evenly without bubbles between the under surface of the slide and the surface of the dark-field condenser.

5. The light should now be centered. This is accomplished while the examiner looks through the low power of the microscope. By focussing up and down, concentric rings of light can be seen and brought to center on the preparation. This centering of the light rings is accomplished by shifting the condenser from side to side by means of the lateral screws, and up and down by means of the vertical condenser screw. By these manipulations the center ring of light becomes a point of light in the middle of the preparation and this adjustment may be necessary because of variations in the thickness of slides. After this has been accomplished, the immersion lens is gently let down until it touches the oil on the top of the cover-glass and adjustment is made with the fine adjustment of the microscope. If a microscope fitted with a dark-field condenser is reserved solely for work of this kind, removing the necessity of changing from the ordinary to the dark-field condenser, and if slides of uniform thickness are employed, centering the light is not ordinarily required but the dark field found by manipulation of the mirror until the proper lighting is secured.

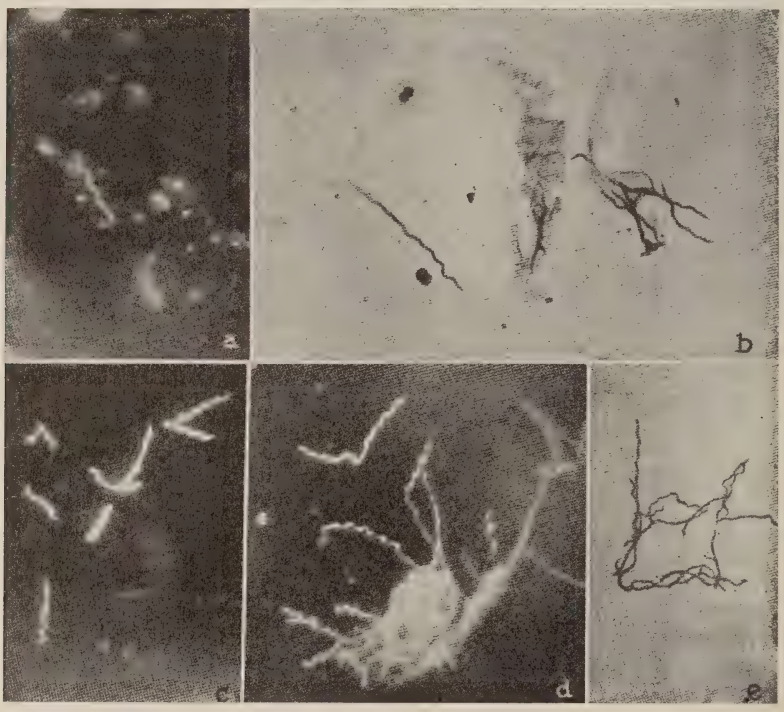

FIG. 20.—SPIROCHAETA REFRINGENS.

From normal genitalia; magnification × 1000. (a) Dark-field view of preparation from smegma. (b) Preparation stained by Fontana's method. (c and d) Dark-field view of culture. (e) Preparation of culture stained by Fontana's method. (Noguchi.)

6. Spirochetes and other formed elements appear as brilliant white objects (Fig. 19). In rabbits with syphilitic scrotal and testicular lesions no difficulty is ordinarily experienced in finding the organisms. In the examination of human lesions and particularly those in the mouth and throat, due care must be exercised against mistaking other spirochetes as *Sp. refrigens* (Fig. 20) *Sp. macrodentium*, *Sp. microdentium*, etc., for *Sp. pallida*.

Technic of Fontana Staining Method for Spirochetes.—While the spirochetes of syphilis and yaws are best detected in the primary lesions by the dark-field method described above, staining methods are occasionally useful

and among the best in my experience is the improved Fontana method as follows:

(1) Films are prepared by placing the material in a drop of distilled water on a slide, spreading and drying in the air. (b) Films may be now fixed by passing over a flame or better by placing in Ruge's fluid for 1 to 5 minutes (glacial acetic acid, 1 c.c.; formalin, 20 c.c. and distilled water, 100 c.c.). (c) The films are now thoroughly washed in running top water when the mordant solution (tannin, 5 grams; phenol, 1 gram and distilled water, 100 c.c.) is poured and warmed until vapor arises for 30 seconds, followed by washing in running water for 15 to 30 seconds. (d) Without drying the silver nitrate solution is applied (silver nitrate, 1 gram; distilled water, 400 c.c. Add ammonia water until slight turbidity results. If excess of ammonia is introduced, the fluid becomes clear again and cannot be used—a trace is all that is required) and heated until steam arises for 30 seconds followed by washing in tap water and drying. If permanent preparations are desired, they must be mounted in xylene balsam; cedar oil causes fading of the spirochetes. The jet-black spirochetes stand out prominently on the clear yellowish background and appear much thicker than when stained by the Giemsa method.

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CHAPTER XXII

SPIROCHETICIDAL PROPERTIES OF DYES

Since the science of chemotherapy began with the investigations of Ehrlich and his collaborators on the action of various dyes in experimental trypanosome infections of mice, it is not surprising that these substances were likewise among the first employed in the experimental chemotherapy of spirochetic infections and especially those transmissible to the smaller animals, as *Sp. obermeieri* infections of mice.

Dyes in the Chemotherapy of Experimental Relapsing Fever.—Vassel[1] was among the first to discover that of the benzidine dyes, trypan-red and amino-naphthol-disulphonic acid were slightly effective in mice infected with the spirochete or spirillum of relapsing fever (*Sp. obermeieri* or *Spirillum recurrentis*). Manteufel[2] also noted slightly curative effects if the dyes were injected at the same time as the organisms. If, however, the infection was given twenty-four hours headway, the dyes had practically no influence upon the infection.

In Hata's[3] investigation the plan was to first determine the highest dilutions of each dye dissolved in alcohol or water and diluted with saline or cane sugar solution, capable of destroying the motility of the organisms in the test-tube as determined by microscopical examinations. As a general rule, loss of motility indicated death of the organisms as injection of the mixtures of blood and dye into mice were generally followed by negative results, or, in some instances, a few spirochetes would appear in the blood of the mice after long periods of incubation.

Animal tests were conducted by infecting mice with *Sp. obermeieri* as described in the preceeding chapter, followed about twenty-four hours later by the subcutaneous or intramuscular injection of the medicament in varying amounts according to body weight. In experiments of this kind with more than 200 dyes of the thiazin and benzidine series and other varieties, the results were generally negative, insofar at least as the animal tests were concerned. Dyes like *methylene-blue* and *methylene-violet* were able to kill *Sp. obermeieri* in the test-tube in dilutions as high as 1:6,000,000 to 1:20,0000,000 but when injected into mice in doses varying from 0.080 gm. to 0.250 gm. per kilo of weight, were without effect upon the development and course of the spirochetosis.

Trypan-red and *trypan-blue*, which as noted in Chapter XI possess well marked trypanocidal properties, are practically devoid of spirocheticidal activity. In Hata's experiments dilutions as low as 1:200 were unable to destroy *Sp. obermeieri* in the test tube and were likewise without effect in mice.

Dyes in the Chemotherapy of Experimental Hen Spirillosis and Rabbit Syphilis.—Hata states that the dyes employed in his investigation had the same effect upon *Sp. gallinarum* in the test tube as upon *Sp. obermeieri*, but the results of animal tests with hen spirillosis were so largely of a negative character as hardly worth mentioning.

These results afford almost complete evidence that the dyes do not possess demonstrable spirocheticidal activity *in vivo*, because of all spirochetic infections hen spirillosis is most easily influenced and cured.

The only experiments conducted with *Sp. pallida* infections were reported by Neisser[4] who treated 86 monkeys at the time of inoculation or a few days afterward with various dyes and with practically negative results in all. Twelve animals received subcutaneous or intravenous injections of *methylene-blue* in dose of 0.2 gm. per animal and eleven about 0.7 gm. by oral administration and all developed syphilis. Eight animals received intravenous infections of *giemsa stain* in dose of 0.4 gm. per animal and all developed the disease. Eight animals received intravenous injections of *fuchsin* in dose of 0.14 gm. per animal and

all developed syphilis; twenty-two additional animals were treated with *parafuchsin* by oral administration in oil and all developed the disease. Eight animals treated with the subcutaneous injection of *trypan-red* in dose of 3 to 5 c.c. of a 2½ per cent. solution developed syphilis.

It may be stated, therefore, that while the dyes are of some importance in relation to the chemotherapy of bacterial and trypanosome infections, little or nothing is to be expected of them in the chemotherapy of syphilis or other spirochetic infections. In other words the various dyes subjected to study including those possessing trypanocidal activity in infected animals, are practically without demonstrable spirochetocidal effects in animals despite the high spirocheticidal activity of some of them in the test tube. It is possible, however, that future researches will show them to possess more importance in the chemotherapy of spirochetosis than now surmized if they can be made to act as the carriers of more active substances into diseased tissues by reason of selective affinity for these.

Mechanism of the Spirocheticidal Activity of Dyes.—Very little is known of the exact mechanism whereby the dyes actually bring about the destruction of spirochetes. *In vitro* the organisms (*Sp. obermeieri* and *Sp. gallinarum*) become slightly stained, lose motility and undergo morphological changes ending in complete solution and total disappearance. As a general rule, complete loss of motility means death, but sometimes such spirochetes are still infective although the incubation period following inoculation before the organisms appear in the blood is apt to be prolonged and the infection less virulent. It would appear therefore, that *in vitro* damage may be done to the proliferative mechanism of the spirochetes from which they but slowly recover.

Probably *in vivo* the spirocheticidal dyes act directly upon the parasites. There is no direct evidence of the production of new compounds in the blood or tissues such as processes of oxidation or reduction with the organic arsenical and antimonyl compounds. However, the fact that some dyes are spirocheticidal *in vivo* and not *in vitro* suggests that a transformation occurs *in vivo* with the production of a new product of the dye or the production of a new combination product of the dye with a tissue constituent.

Influence of Dyes upon the Development of Immunity in Spirochetosis. As stated in Chapter XI Ehrlich and Shiga observed in 1904 that after the cure of *Tr. brucei* infections of mice with trypan-red and other dyes, the mice were refractory to re-infection with this strain for about ten days. Terry and others confirmed these observations and showed that this temporary immunity was not due to the presence of unexcreted dye.

Does a similar immunity develop in mice or fowl after the cure of experimental *Sp. obermeieri* and *Sp. gallinarum* infections? Investigations along these lines have been hampered by the fact that in both infections an immunity develops independent of drug treatment which tends to become stronger with each relapse.

Working with *Sp. novyi* in mice, I have found that infected mice cured with a single dose of trypan-red are more difficult to re-infect during the following fifteen days than untreated control mice during their first relapse. Mice treated with dye alone could be infected 60 hours later with the same amount of spirilli-blood as control mice showing that the influence of unexcreted medicament does not last longer than this time, when an ordinary curative dose is given by intraperitoneal injection. Hata states that mice cured by a single injection of a dye early in the course of relapsing fever are susceptible to re-infection about 60 days later, but require a larger number of spirochetes (*Sp. obermeieri*) for infection than control mice to overcome the immunity. This indicates that the immunity does not last longer than 60 days and I believe it is of much shorter duration when complete cure has been effected. In case of incomplete cure the animals are highly refractory to re-infection, but this is due to the presence of living spirochetes in the tissues even though the blood is apparently free, and not to any influence on the part of the dye.

I believe it may be stated, therefore, that the administration of curative doses of trypan-red and presumably of other spirocheticidal dyes, aids in engendering a state of temporary resistance or immunity to re-infection. This is probably not due to the influence of the dyes upon the antibody producing tissues, because investigations in trypanosomiasis along these lines have yielded negative results. Rather the dyes release from spirochetes by reason of their spirocheticidal activity, active antigenic principles which engender the production of the immune substances.

Furthermore, there is no reason for believing that this immunological process has any influence upon the cure of experimental spirochetosis by single large doses of any dye; *therapie magna sterilisans* is due to the direct action of the dye or its products upon the spirochetes because there is not sufficient time for antibody production. But in the treatment of chronic infections with repeated doses of a dye compound it is entirely likely that the production of immune principles aid in bringing about a cure.

Acquired Resistance of Spirochetes to Dyes.—As discussed in Chapter XI trypanosomes readily acquire resistance to various dyes and especially parafuchsin and trypan red when infected mice or rats are treated with sub-curative doses of these compounds. The surviving trypanosomes become resistant to the dye and this "fastness" may be increased by additional treatments and transfers, the state of resistance being transmissible.

Similar investigations with the spirochetes does not appear to have been reported. Several years ago I worked with *Sp. novyi* and trypan-red and after seven transfers thought that the strain had acquired a definite increased resistance to this due, but owing to the irregularities of relapsing fever infections of mice the experiments were not as definite and decisive as similar experiments in trypanosomiasis. However, a conservative estimate of the experiments has lead me to the conclusion that *Sp. novyi* may acquire a "resistance" or "fastness" to dyes and it is highly probable that the same is true of other spirochetes.

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CHAPTER XXIII

TOXICITY AND SPIROCHETICIDAL PROPERTIES OF ARSENICAL COMPOUNDS

Shortly after the discovery of the trypanocidal activity of atoxyl, Uhlenhuth and his associates conducted similar studies with atoxyl and mercury in the chemotherapy of spirochetic infections. This was quickly followed by the investigations of Ehrlich, Hata and others, who derived great encouragement by the fact that some new arsenical compounds without demonstrable trypanocidal properties were found to favorably influence spirochetic infections and especially relapsing fever of mice and spirillosis of chickens. From the standpoint of practical results, therefore, studies in the chemotherapy of

organic arsenical compounds in experimental spirochetic infections have been among the most fruitful.

Arsenical Compounds in the Chemotherapy of Experimental Relapsing Fever.—Most experimental work has been conducted with mice infected with *Sp. obermeieri* (*Sp. recurrentis*), the cause of European relapsing fever; *Sp.duttoni* (African relapsing fever) and *Sp. novyi* (American relapsing fever) have been less frequently employed and *Sp. carteri* (relapsing fever of India) does not appear to have been used at all.

In practically all of the early work conducted by Hata in collaboration with Ehrlich, mice were employed, the maximum tolerated and minimum curative doses being determined by injecting the medicaments subcutaneously. As a general rule, the drug was administered 24 hours after the intraperitoneal injection of *Sp. obermeieri*, although the preventive or prophylactic activity of many of the compounds was likewise determined by giving the drug 24 to 48 hours or more before infection.

In my experiments such inorganic compounds of arsenic as *arsenious acid*, *sodium arsenate* and *sodium arsenite* had no influence at all upon the course of *Sp. novyi* infections of mice when these compounds were administered by subcutaneous injection and in doses as large as the maximum tolerated amounts.

The effects of organic arsenical compounds in general upon *Sp. obermeieri* infections of mice are much less powerful than those produced by these compounds upon *Tr. equiperdum* infections of rats. Even *arsphenamin* (*salvarsan*) has an index of but 2.7 for *Sp. obermeieri* infections as compared with an index of 15 or higher for various trypanosome infections.

The pentavalent compounds are much less spirocheticidal in these infections than the trivalent compounds. In trypanosomiasis some pentavalent compounds and especially derivatives of the arsonic acids, as amino-oxyphenyl arsonate and tryparsamid,[1] are highly trypanocidal, but these as well as atoxyl and arsacetin are much less effective in experimental relapsing fever.

Best results have been observed with the arsenobenzene compounds, as *arsphenamin* and *neoarsphenamin*. The chemotherapeutic index of arsphenamin is as follows, the compound being administered to the mice by subcutaneous injection:

$$\frac{\text{Maximum tolerated dose: 0.170 gm. per Kilo}}{\text{Minimal curative dose: 0.06 gm. per Kilo}} = 2.8$$

The chemotherapeutic index of neoarsphenamin is about as follows:

$$\frac{\text{Maximum tolerated dose: 0.350 gm. per Kilo}}{\text{Minimal curative dose: 0.012 gm. per Kilo}} = 2.9$$

Neoarsphenamin, therefore, tends to have a slightly higher index than arsphenamin and this is true in experimental trypanosomiasis. It may be noted in passing that the percentage of arsenic in the different compounds bears no constant relation to either toxicity for the mice or curative activity.

The cure of experimental relapsing fever of mice and rats is, therefore, more difficult than the cure of trypanosome infections and likewise, as will be shortly shown, more difficult than the cure of *Sp. gallinarum* infections of chickens and *Sp. pallida* infections of rabbits. Of course, small amounts of the various medicaments may suffice for temporarily removing *Sp. obermeieri* and *Sp. novyi* from the blood of mice, but the amounts required for effecting complete cure, as determined by re-infecting the mice 60 days later, are much higher. In general terms, the cure of experimental relapsing fever

of mice is more difficult to effect than the cure of experimental trypanosomiasis or other spirochetic infections of the lower animals and affords an additional example of the important influence exerted by the parasite employed upon chemotherapeutic investigations.

Arsenical Compounds in the Chemotherapy of Experimental Chicken Spirillosis.—Probably the first experimental spirochetic infection to be treated with an arsenical compound was hen spirillosis (*Sp. gallinarum*) with *atoxyl* by Uhlenhuth, Gross and Bickel[2] in 1907.

These investigators determined that the lethal dose for chickens by intramuscular injection was about 0.5 gm. per kilo of weight. As a general rule the injection of 0.05 gm. per kilo proved curative even when injected 48 hours after infection and when numerous spirochetes were present in the peripheral blood. Likewise, when this amount was injected before or simultaneously with the spirochetes, infection did not usually take place. When administered by mouth 0.1 gm. per kilo also proved curative.[3]

A little later Uhlenhuth and Manteufel[4] combined atoxyl and mercury in order to permit the treatment of human syphilis simultaneously with arsenic and mercury; this *mercuric arsanilate* was found to contain 31.65 per cent. mercury and 23.75 per cent. arsenic. The compound was more toxic than atoxyl and the curative dose remained about the same.

Following these investigations Hata determined the toxicity of a number of new arsenical compounds for the chicken by intramuscular injection and likewise their curative activity in *Sp. gallinarum* infections. In a general manner it was noted that the maximum tolerated doses of the various compounds was slightly greater for chickens by intramuscular injection than for mice by subcutaneous injection and that the curative activity of all compounds was very much greater for *Sp. gallinarum* than for *Sp. obermeieri.* Even atoxyl, which possesses very little prophylactic and curative value in *Sp. obermeieri* infections, is distinctly curative for *Sp. gallinarum* infections, yielding a chemotherapeutic index of about 5. Forneau and Levaditi[5] and Forneau[6] have reported that some of the *derivatives* of *phenylarsonic* and *arylarsonic acids* possess well-marked spirocheticidal activity, yielding chemotherapeutic indices of 5.

Jeanselme and Pomaret[8] have likewise reported that some of these phenylarsonic acid derivatives possess curative activity in hen spirillosis and rabbit syphilis; according to the latter a compound designated as 132 or *amino-arsenol phenol* is tolerated by chickens in dose of 0.150 gm. per kilogram of weight and is curative for *Sp. gallinarum* infections in dose of 0.0037 gm. per kilogram, yielding, therefore, an index of 40.

Doubtless these favorable results have been greatly aided by a marked tendency for the development of immunity and spontaneous cure in hen spirillosis. While this experimental infection is serviceable for eliciting minor degrees of spirocheticidal activity of new compounds, great care is required in the interpretation and evaluation of the results.

As is true in the treatment of trypanosomiasis and *Sp. obermeieri* infections, trivalent organic compounds of arsenic (arsphenamin and neoarsphenamin) are more active than pentavalent compounds; according to Sezary and Pomaret,[9] the curative activity of arsphenamin is 5 times and neoarsphenamin 3.3 times stronger in *Sp. gallinarum* infections than Fourneau's pentavalent compound acetyl-oxyamino-phenylarsinic acid,[190] although, when these substances are given orally instead of being injected intramuscularly or intravenously, the differences in curative activity are much less.

Arsenical Compounds in the Chemotherapy of Experimental Syphilis.—Owing to the larger size and expense of rabbits, the necessity of using a number of them for determining the toxicity and minimal curative doses of any medicament and the relatively long times required for the evaluation

of the therapeutic results, rabbit syphilis has not been as extensively employed in experimental chemotherapy as trypanosome infections. The earliest work in experimental syphilis was conducted with monkeys and necessarily was on a very limited scale. In rabbits the earliest chemotherapeutic investigations were conducted with experimentally produced syphilitic keratitis, but testicular lesions are now employed almost exclusively.

In many instances investigators have expressed the spirocheticidal activity of medicaments on the basis of rapidity of disappearance of *Sp. pallida* from the local lesions; as stated in the preceding chapter, however, the dose required to bring about these results may not be necessarily the curative dose, as spirochetes may still be found in the lymphatic glands. It is necessary, therefore, to examine the lymph-glands for spirochetes by additional animal-inoculation tests requiring more animals and still further delaying the time when the results of final tests will be known. Despite these economic and technical difficulties, the treatment of rabbit syphilis constitutes in the final analysis an almost necessary part of any chemotherapeutic investigation in syphilis.

Spirocheticidal Activity of Inorganic Compounds of Arsenic in Syphilis.—In *Sp. pallida* infections of monkeys Neisser[10] has observed that arsenious acid was without curative activity. Fifty-six monekys weighing roughly from 2 to 6 kilograms received intravenous injections of doses varying from 0.011 to 0.089 gm. per animal within 2 to 4 days after inoculation or after the development of the primary lesion; in practically no instance was syphilis prevented and at best but slight curative properties were apparent.

Toxicity and Curative Activity of Atoxyl (Sodium Arsanilate) in Experimental Syphilis.—From the historical standpoint, *atoxyl* is of great interest in the treatment of syphilis because it was the first organic compound of arsenic to be used for the prophylactic and curative treatment of experimental syphilis of monkeys and rabbits.

Metchnikoff[11] showed in 1907 that atoxyl in dose of 0.3 to 0.5 gm. per kilo of weight prevented syphilis of apes if administered within 15 days of the time of inoculation. Similar results were observed by Salmon[12] with intramuscular injections of atoxyl in dose of 0.05 to 0.1 gm. per kilo.

Uhlenhuth, Hoffman and Roscher[13] observed the curative value of 10 to 25 doses of atoxyl in amounts of 0.05 to 0.1 gm. injected intramuscularly every 2 or 3 days in syphilitic apes and rabbits, and, in experiments conducted with Weidanz,[14] found this compound superior to mercuric chlorid in the treatment of syphilitic keratitis of rabbits. Levaditi and Yamanouchi[15] also found that atoxyl in dose of about 0.1 gm. per kilo injected subcutaneously prevented the development of syphilitic keratitis in rabbits. These results lead to the use of atoxyl in the treatment of human syphilis, until it was discovered that the compound produced changes in the optic nerve with disturbances of vision.

As a matter of fact, the curative activity of single doses of atoxyl in scrotal or testicular syphilis of rabbits is not very high. In my experiments, the maximum tolerated dose of pure compounds for rabbits by intravenous injection was about 0.120 gm. per kilo and the minimal curative dose varied from 0.030 to 0.060 gm. per kilo, yielding chemotherapeutic indices of 2 to 4. Smaller doses sufficied for reducing the numbers of spirochetes in the lesions with subsidence of the inflammatory changes, but only maximum amounts were curative in single doses.

Neisser[16] in 1909–1910 administered to 47 monkeys by intravenous or subcutaneous injection of 0.05 to 1.0 gm. per animal, at the same time as inoculation with syphilis virus; of these, 4 developed primary lesions and 43 did not. Neisser also treated 63 additional animals with atoxyl in dose of 0.05 to 1.8 gm. per animal (2 to 6 kilograms) after the development of primary lesions; of these, 25 were not cured, as after the lesions disappeared re-inoculation was positive in all. Thirty-six animals were probably cured as re-inoculations were negative, while the results were doubtful with two animals.

Toxicity and Spirocheticidal Activity of Arsenophenylglycin, Arsacetin and Allied Compounds in Experimental Syphilis.—Ehrlich and Hata did not employ experimental syphilis on a large scale in their chemotherapeutic investigations. Most of the curative tests were conducted with mice infected with *Tr. brucei* or *Sp. obermeieri* and chickens with *Sp. gallinarum.*

Hata found the curative activity of *arsenophenylglycin* for scrotal syphilis of rabbits to be about 0.15 gm. per kilo by intravenous injection, and since this trivalent arsenical is tolerated in dose of about 0.200 gm. per kilo, the chemotherapeutic index is only 1.6, although at one time it was thought worthy of trial in the treatment of human syphilis. Neisser[17] treated 18 monkeys with 3 to 7 injections of arsenophenylglycin, totalling 0.15

to 0.7 gm. per animal, at the same time as inoculation with syphilis virus; of these, only one developed a primary lesion while 17 escaped. Three animals were treated with 0.3 gm. and 5 more with arsenophenylglycin plus 0.1 gm. atoxyl eight days after inoculation; all escaped syphilis. Thirty-five monkeys were treated after the development of primary lesions with 0.05 to 0.9 gm. per animal; of these, 29 were probably cured. Neisser observed the rather high toxicity of this compound for both monkeys and human subjects. Neisser also treated 24 monkeys with 0.2 to 0.5 gm. of *arsacetin* per animal at the time of inoculation with virus; of these, 2 developed syphilis and 22 escaped. Of fourteen additional animals treated with 0.35 to 0.7 gm. per animal after the development of syphilis, cure was effected in ten as re-inoculation was positive, whereas in the remaining four animals re-inoculation was doubtful or negative and indicative of failure of cure.

Sixteen monkeys were treated with *sodium-p-oxy-phenylarsenate* (*phenarsol*); the dose was 0.05 to 0.39 gm. per kilogram injected 3 or 4 days to several weeks after inoculation. Twelve animals survived and of these 9 developed primary lesions and 3 escaped. Of 4 additional animals treated with 0.05 to 0.36 gm. per kilogram after the primary lesions had developed, 2 were cured as shown by subsequent re-inoculation.

Seven monkeys were treated with *sodium-p-carbamino-arsenate* ("*asuran*"); the doses were 0.125 to 0.269 gm. per kilogram given about the time of inoculation; 6 escaped infection while 1 developed a primary lesion.

Seven monkeys were treated with *sodium acetyl-anthranilarsenate* ("*arsanthran*"); the doses were 0.1 to 0.48 gm. per kilogram about 3 to 4 days after inoculation; 6 developed primary lesions and 1 escaped. Three more animals treated after the development of primary lesions were not cured.

Levaditi and Navarro-Martin[18] treated syphilitic rabbits with sodium salt of *acetyl-oxyphenylarsonic acid* (189); the maximum tolerated dose was 0.4 gm. per kilogram by subcutaneous injection, while 0.175 gm. was curative, yielding a chemotherapeutic index of 1:4.

Toxicity and Spirocheticidal Activity of Arsphenamin (Salvarsan) in Experimental Syphilis.—Hata observed that the minimal curative dose of *salvarsan* (*arsphenamin*) for scrotal syphilis of rabbits was from 0.010 to 0.015 gm. per kilo and the investigations of Kolle[19] and myself have amply confirmed this work. Much smaller doses suffice for causing the spirochetes to disappear from the local testicular lesions; Nichols,[20] for example, found 0.0045 gm. per kilo sometimes sufficient for this purpose, but amounts less than 0.010 gm. per kilo are seldom sufficient for a permanent cure with sterilization of the lymph-glands.

Kuznitsky,[21] working with Neisser, treated 2 monkeys with subcutaneous injections of 0.025 gm. per kilogram of salvarsan in alkaline solution, several days before inoculation; both of these animals developed primary lesions. Three more animals were treated with 0.025 gm. per kilogram in neutral suspension at the time of inoculation and all escaped infection. Ten more animals were treated 2 to 223 days after the development of primary lesions with 0.025 gm. per kilogram in alkaline solution by intramuscular injection; 5 were apparently cured, as re-inoculations were successful, while 3 were not cured and the results doubtful in 2. Of 2 additional animals treated with 0.015 gm. per kilogram by intravenous injection, 1 was cured (re-inoculation positive) and 1 was not cured (re-inoculation negative).

According to Hata and Kolle the chemotherapeutic index of salvarsan is about 10 expressed as follows:

$$\frac{\text{Maximum tolerated dose: 0.100 gm. per Kilo}}{\text{Minimal curative dose: 0.010 gm. per Kilo}} = 10$$

With American-made arsphenamin (salvarsan) I have found, however, that the toxicity is generally less (0.110 to 0.0140 gm. per kilo) and the curative activity may be slightly greater. Some lots have been curative in amounts as low as 0.006 gm. per kilo, although the majority have required the administration of 0.010 to 0.012 gm. per kilo to effect complete sterilization of the lymphatic system. The chemotherapeutic indices of American-made arsphenamin may vary, therefore, from 10 to 20.

Hata also determined the spirocheticidal activity of "*arsenoxid*" which is supposed to form by oxidation in solutions of arsphenamin; while this substance is 6 to 7 times more toxic than arsphenamin, it is only about 3 to 4 times more spirocheticidal (0.003 gm. per kilo), so that its chemotherapeutic index of 5 is from two to four times less than that of arsphenamin.

Toxicity and Spirocheticidal Activity of Neoarsphenamin (Neosalvarsan) in Experimental Syphilis.—Kolle found the minimal curative dose of *neoarsphenamin* in rabbit syphilis to be about 0.015 gm. per kilogram. In my experiments the maximum tolerated dose of American-made neoarsphenamin (neosalvarsan) for rabbits has been about 0.350 gm. per kilo; the minimal curative doses have varied from 0.008 to 0.020 gm. per kilo and yielding, therefore, chemotherapeutic indices of 15 to as high as 40. These results are of considerable significance, for they indicate a greater range between toxic and curative doses than is observed with arsphenamin, as has been found true likewise in experimental trypanosomiasis.

Sodium arsphenamin (sodium salvarsan), which for a time enjoyed a brief popularity in the treatment of human syphilis, is borne by rabbits in about 0.210 gm. per kilo by intravenous injection. The minimal curative dose is about 0.020 gm. per kilo, yielding a chemotherapeutic index of about 10, which is about equal to that of arsphenamin but distinctly less than that of neoarsphenamin.

Toxicity and Spirocheticidal Activity of Silver and Other Arsphenamins in Experimental Syphilis.—Within the past few years Kolle has produced a *silver arsphenamin* (*silver salvarsan*) which he regards as having a higher curative activity than arsphenamin. In his experiments the average minimal curative dose has been about 0.004 gm. per kilo as compared with 0.010 gm. of arsphenamin. In my experiments the maximum tolerated dose of American-made silver arsphenamin for rabbits has varied from 0.090 to 0.110 gm. per kilo and the minimal curative doses from 0.004 to 0.010 gm. Some lots of silver arsphenamin are, therefore, more spirocheticidal than arsphenamin and when the toxicity is low the chemotherapeutic index may be somewhat higher (11 to 27) than the index for arsphenamin (10 to 20). It is to be noted, however, that the index for silver arsphenamin is not higher than the average index of neoarsphenamin (neosalvarsan) of American manufacture (15 to 40).

Kolle also found that *copper*, *gold* and *platinum arsphenamins* were more spirocheticidal (0.004 to 0.005 gm. per kilo) than arsphenamin (0.010 gm. per kilo), but usually slightly less spirocheticidal (0.004 gm. per kilo) than silver salvarsan; the former compounds have not been employed in the treatment of human syphilis. Danysz has prepared a compound of arsphenamin with antimony and silver designated as "*luargol*" or No. 102; according to Bonard,[22] it was found by Danysz to be borne by rabbits in dose of about 0.200 gm. per kilo with a minimal curative dose as low as 0.003 gm. per kilo and yielding, therefore, a chemotherapeutic index as high as 60. The one sample of this compound which I was able to test was borne by rabbits in dose of about 0.140 gm. per kilo, with a minimal curative dose of 0.016 gm., yielding an index of about 9. I believe that the differences in our results are largely due to the fact that the smaller dose employed by Danysz may be sufficient for sterilizing the testicular lesions but that much more is required to effect complete sterilization of the lymphatic glands.

Toxicity and Spirocheticidal Activity of Sulpharsphenamin in Experimental Syphilis.—Recently Voegtlin, Johnson and Dyer[23] described the preparation, chemical and biological properties of *sulpharsphenamin*, which may be injected subcutaneously as well as intravenously. The maximum tolerated

dose for rats has been reported by them as 0.400 to 0.500 per kilo, but the toxicity for rabbits has not been stated. The minimal curative dose for rabbits has been given as 0.015 gm. per kilo, although it is not clear in their paper whether or not this amount served to sterilize the lymphatic glands as well as the testicular lesions. In my experiments the maximum tolerated dose of sulpharsphenamin for rabbits by intravenous injection was about 0.300 gm. per kilo and the minimal curative dose, including sterilization of the lymphatic glands, about 0.022 gm. per kilo, yielding a chemotherapeutic index of about 14. By subcutaneous injection the maximum tolerated dose was about the same; the minimal curative dose was the same or slightly less, being from 0.018 to 0.024 gm. per kilo. This substance possesses, therefore, a chemotherapeutic index equal to or higher than that of arsphenamin, and equal to some lots of neoarsphenamin. A distinct advantage is the possibility of administering the compound to some human subjects by subcutaneous or intramuscular injection in the treatment of syphilis.

Toxicity and Spirocheticidal Activity of Sodium Cacodylate, Tryparsamid and Other Pentavalent Organic Arsenical Compounds.—With the exception of sodium arsanilate (atoxyl), all of the organic arsenical compounds discussed above are trivalent organic compounds of arsenic. The pentavalent compounds are much less curative for rabbit syphilis, just as the majority of them are distinctly less curative for experimental relapsing fever and trypanosomiasis.

Sodium cacodylate, which at one time had considerable popularity in the treatment of syphilis, has been found by Nichols[24] without curative activity in rabbit syphilis, even in amounts as large as the maximum tolerated dose. Runnels[25] found that the compound was borne by half-grown rabbits in dose of about 0.3 gm. per kilogram of weight; according to Nichols the maximum tolerated dose by intravenous injection is from 0.150 to 0.200 gm. per kilo.

Neisser[26] treated 19 monkeys with subcutaneous injections of doses of about 0.8 gm. per kilogram several days after inoculation with virus; 15 developed syphilis. Eighteen additional animals were treated with doses as high as 1.0 gm. per kilogram after the development of lesions, but none were cured. Neisser concludes his account by quoting Ehrlich who said, "The preparation is indeed non-toxic, but it is also therapeutically worthless."

Nichols[27] also found that *disodium ethyl arsonate* ("mon arsone") was without appreciable spirocheticidal activity in rabbit syphilis in amounts as large as the maximum tolerated dose by single intravenous injection.

Tryparsamid, which possesses a well-marked degree of trypanocidal activity, was found by Brown and Pearce[28] and myself to have a low spirocheticidal value. This substance is remarkably low in toxicity, being borne by rabbits in doses as high as 1.0 gm. per kilo, but the minimal curative dose required for complete sterilization of the testicles and lymphatic glands is as high as 0.3 to 0.5 gm. per kilo with a chemotherapeutic index as low, therefore, as 3.

Recently French investigators revived interest in the *phenyl arsonic acids* and especially in the sodium salt of amino-oxyphenyl arsonic acid ("189"). Levaditi and Martin[29] and Fourneau, Levaditi and their colleagues[30] have found this pentavalent compound borne by rabbits in doses as high as 0.250 gm. per kilo with a minimal curative dose of 0.100 to 0.150 gm. per kilo. Fourneau[31] states that the neurotropism of atoxyl may have been due to the presence of impurities and that the whole group of phenylarsonic acids are worthy of further study in the treatment of trypanosomiasis and spirochetosis.

Comparison of the Trypanocidal and Spirocheticidal Properties of Arsphenamin and Neoarsphenamin.—As stated in Chapter XII trypanocidal tests have proven of distinct value in evaluating the properties of different lots of arsphenamin and neoarsphenamin for the treatment of human syphilis. Not all trypanocidal arsenical compounds, however, possess corresponding spirocheticidal properties; some, like tryparasamid, are much more trypanocidal than spirocheticidal and others are more spirocheticidal than trypanocidal.

Comparative tests with different lots of arsphenamin and neoarsphenamin have shown, however, that the relation between trypanocidal and spirocheticidal properties is only broad and general as shown in Table 4.

TABLE 4.—A COMPARISON OF THE TRYPANOCIDAL AND SPIROCHETICIDAL PROPERTIES OF ARSPHENAMIN AND NEOARSPHENAMIN

COMPOUND	TRYPANOCIDAL TESTS (RATS)			SPIROCHETICIDAL TESTS (RABBITS)		
	MAXIMUM TOLERATED DOSE	MINIMAL CURATIVE DOSE	INDEX	MAXIMUM TOLERATED DOSE	MINIMAL CURATIVE DOSE	INDEX
Arsphenamin (A)	0.100	0.004	1:25	0.090	0.012	1:8
Arsphenamin (B)	0.140	0.008	1:17	0.120	0.006	1:20
Arsphenamin (C)	0.140	0.003	1:46	0.110	0.006	1:18
Silver arsphenamin	0.130	0.002	1:65	0.115	0.006	1:19
Neoarsphenamin (A)	0.260	0.006	1:43	0.250	0.008	1:31
Neoarsphenamin (B)	0.250	0.006	1:41	0.250	0.010	1:25
Neoarsphenamin (C)	0.260	0.006	1:43	0.240	0.010	1:24
Neoarsphenamin (D)	0.200	0.008	1:25	0.180	0.008	1:22
Neoarsphenamin (E)	0.250	0.002	1:125	0.250	0.012	1:21
Neoarsphenamin (F)	0.230	0.010	1:23	0.200	0.012	1:17

The toxicity of each compound was determined by intravenous injection into rats and rabbits, the table showing the maximum tolerated amounts per kilo of body weight. The trypanocidal tests were conducted according to my method with rats infected with *Tr. equiperdum* and the table shows the smallest amounts per kilo effecting complete sterilization. The spirocheticidal tests were conducted with rabbits infected intratesticularly with *Sp. pallida*, treatment being given 3 to 4 weeks later; the tables show the smallest amounts required for complete sterilization not only of the testicles but of the inguinal lymph-glands as well. In this connection it should be stated that these tests were conducted with compounds prepared by one laboratory during 1922 and at a time when their trypanocidal properties were particularly good.

Insofar as the toxicity of arsphenamin and neoarsphenamin is concerned, there is a fairly definite relation between the maximum tolerated doses of both compounds for the rat and rabbit. Sometimes the maximum tolerated dose for the rabbit is slightly less than that for the rat, but the differences are not great.

In every instance the chemotherapeutic index of trypanocidal activity was higher than the index of spirocheticidal activity; in other words arsphenamin and neoarsphenamin are more trypanocidal than spirocheticidal.

The relation between trypanocidal activity in the rat and spirocheticidal activity in the rabbit is not, however, definite or constant. Of course, it is

to be remembered in this connection that, while the trypanocidal activity of a compound can be determined within sharply defined limits, it is not readily possible to accomplish this in spirocheticidal tests. In a broad and general way the minimal spirocheticidal dose of arsphenamin (0.008 gm. per kilo) is from 2 to 3 times more than the minimal trypanocidal dose (0.003 gm. per kilo). The same is true of neoarsphenamin, since the spirocheticidal dose (0.012 gm. per kilo) is from 2 to 3 times more than the trypanocidal dose (0.006 gm. per kilo). Individual exceptions, however, are not lacking, a striking example being afforded by neoarsphenamin (E) in Table 4, which was trypanocidal in dose of but 0.002 gm. per kilo, but spirocheticidal only in amounts as high as 0.012 gm. per kilo.

Arsenical Compounds in the Treatment of Venereal Spirochetosis of Rabbits.—As described in Chapter XXI, several European and American investigators have described a disease of the penis and vulva of rabbits caused by *Sp. cuniculi* (Noguchi). According to Levaditi, Marie and Isaien[32] the spirochete is not infectious for man.

According to Noguchi,[33] the intravenous injection of arsphenamin in dose of 0.02 gm. per kilogram of body weight, resulted in the destruction of the spirochetes in the lesions in twenty-four hours. The lesions healed promptly and completely in seven days, and no recurrence was observed during a period of five months.

Kolle and Ruffer[34] found that it required the intravenous injection of 0.005 to 0.007 gm. silver arsphenamin to cure the lesions while 0.004 gm. per kilo was sufficient for the cure of the primary *Sp. pallida* lesions. In rabbits infected with both *Sp. pallida* and *Sp. cuniculi*, a dosage of silver arsphenamin of 0.004, 0.005 or 0.006 gm. per kilo body weight caused *Sp. pallida* to disappear and the chancre to heal, while the *Sp. cuniculi* still persisted in flat erosions. By this chemotherapeutic test they have furnished an additional method for the differentiation of the spirochetes and lesions.

Arsenical Compounds in the Chemotherapy of Experimental Yaws (Frambesia Tropica).—Experimental yaws of the rabbit is apparently more easily cured by spirocheticidal agents than experimental syphilis. As stated in Chapter XXI, the disease can be produced in rabbits by inoculation of the testicles with material from human cases of yaws or from experimentally produced lesions of monkeys or rabbits. Apparently *Sp. pertenuis* is not as virulent as *Sp. pallida* for rabbits; the spirochetes do not appear to be as widely distributed and there is a greater tendency for the development of immunity and spontaneous recovery. For these reasons the disease is more readily influenced by drugs and responds more promptly to arsphenamin and neoarsphenamin than the testicular and scrotal lesions of syphilis.

Nichols[35] was apparently first to discover the curative effects of arsphenamin in yaws of the rabbit. The intravenous injection of 0.0045 gm. per kilogram of body weight resulted in the permanent cure of four rabbits with well-developed testicular lesions, while, out of 5 rabbits infected with *Sp. pallida* and treated with a similar dose, 3 relapsed in 31, 36 and 49 days respectively. Castelli[36] subsequently confirmed the high curative activity of salvarsan and neosalvarsan in testicular and scrotal yaws of the rabbit, finding that the latter in doses of 0.015 to 0.035 gm. per kilogram by intravenous injection served for the sterilization of these local lesions in 3 to 6 days. Schamberg and Klauder[37] failed to cure the scrotal lesions of yaws in rabbits with 0.005 gm. of arsphenamin per kilo, but observed that the curative dose of neoarsphenamin was 0.008 gm. per kilo. No one, however, has yet reported upon the presence of *Sp. pertenuis* in the lymph-glands of rabbits; it is highly probable that in experimental yaws the spirochetes gain access to these tissues, just as *Sp. pallida* does in experimental syphilis, and if so, the amounts of salvarsan and neosalvarsan required for complete sterilization would be larger than reported above for the healing of the testicular, scrotal or other external lesions with disappearance of spirochetes.

An abundance of clinical data shows that the curative properties of arsphenamin and neoarsphenamin are even more striking in dissipating the external lesions of yaws in human beings than the lesions of syphilis; but I shall discuss this subject in more detail in a later chapter.

Mechanism of Spirocheticidal Activity of Arsenical Compounds.—Soon after the discovery of the trypanocidal and spirocheticidal (for *Sp. gallinarum*) properties of atoxyl, the mechanism of its action in living animals commanded considerable investigation. In 1907, Uhlenhuth and Gross[38] thought that atoxyl inhibited the multiplication of *Sp. gallinarum* and brought about their destruction by stimulating the antibody producing tissues and particularly by facilitating phagocytosis. Similar views were expressed by Aoki,[39] in 1914, investigating the influence of atoxyl upon antibody production in hen spirillosis.

Levaditi and McIntosh[40] and later Levaditi and Yamanouchi,[41] noting that atoxyl was practically without toxicity for *Sp. gallinarum in vitro* but curative *in vivo*, advanced the theory that the compound first underwent a change *in vivo*, in which the liver was intimately involved, with the production of spirocheticidal substances. The mechanism was regarded the same as occurring in the cure of trypanosome infections; according to Levaditi[42] atoxyl is reduced primarily in the liver and this product then unites with its proteins to form "trypanotoxyl," the active trypanocidal agent. Gonder,[43] working in Ehrlich's laboratory, then showed that while solutions of salvarsan *in vitro* were able to reduce the motility of *Sp. gallinarum* and various trypanosomes, infectivity was not destroyed. However, if salvarsan was administered to fowls and a little later blood drawn, the serum secured and mixed with blood containing *Sp. gallinarum in vitro*, the injection of such mixtures in rice birds was not followed by infection, suggesting that some change had occurred in the serum with the production of more active spirocheticidal compounds. Recently, Lee[44] advanced a somewhat similar hypothesis regarding the mechanism of the spirocheticidal activity of arsphenamin and neoarsphenamin in syphilis by stating that these medicaments united with the proteins of cells about the foci of infection and that these cellular-protein-arsphenamin compounds were the active spirocheticidal substances.

Ehrlich has long maintained that atoxyl was reduced in the animal to an active trivalent compound and that the latter explained why atoxyl was active *in vivo* and not *in vitro*. Ehrlich believed that the power of reducing arsenical compounds varied in animals of different species, but was more or less constant for the animals of any one species and that this explained the varying effects of atoxyl in animals of different species analogous to the different oxidizing activities of different microörganisms.

Continuing this line of thought, Ehrlich and Bertheim showed that the oxidation of salvarsan *in vitro*, with the formation of "arsenoxid," increased toxicity 7 to 10 times for mice and rabbits, and at the same time increased trypanocidal and spirocheticidal activity. Ehrlich believed that the same process occurred in man and the lower animals and that these oxidation products constituted the active spirocheticidal substances. According to Ehrlich, the pentavalent compounds first require reduction to the more parasitropic trivalent substances and the increase of spirocheticidal activity of atoxyl in an emulsion of liver cells noted by Levaditi was due to a reduction *in vitro*. According to Friedberger, a similar reduction of atoxyl can be brought about by thioglycol acid which Bertheim explains by the primary production of "arsenoxid" followed by union with the thioglycol acid and the production of *p*-amino-phenyl-arsenthioglycol acid.

I have previously discussed in more detail the mechanism of trypanocidal activity of pentavalent and trivalent organic compounds of arsenic, calling attention to the work of Voegtlin and Smith indicating that arsphenamin and neoarsphenamin are probably not trypanocidal as such, but first require oxidation either *in vitro* or *in vivo*. While similar experiments have not been conducted with *Sp. pallida*, I think it is permissible to explain the spirocheticidal activity of these substances on the same basis. The weight of experimental data strongly supports the hypothesis that the organic compounds of arsenic must first undergo the processes of oxidation or reduction, according to the nature of the compound, before toxicity for parasites is obtained. In this connection I may mention however, that Schumacher[45] has recently advanced the hypothesis that the arsenobenzols destroy spirochetes and other organs by abstracting oxygen from them. His experiments were not conducted with *Sp. pallida* but by histo-chemical studies with other organisms and cells to show these effects.

As will be discussed immediately, there is evidence to support the theory that the curative activity of arsphenamin and neoarsphenamin in syphilis may be aided by antibody production when the drugs are repeatedly given. But cures effected by single large doses are probably due entirely to the "oxids," since there is no time for antibody production; but just how these "oxids" bring about the destruction of *Sp. pallida* and other spirochetes and trypanosomes is not known. Apparently these substances unite with the protoplasm by means of sulphydril compounds. Probably the primary effect is an actual increase in the numbers and activity of the attacked parasites, as indicated by the investigation of Frei,[46] followed by protoplasmic changes and death.

Furthermore, as shown by Voegtlin and Thompson,[47] the rate of excretion of the arsenical compound has an important influence upon its parasiticidal activity, the directly toxic trivalent oxid compounds showing the slowest rate, the pentavalent arsenicals the fastest, and the arsenobenzene compounds occupying an intermediate position. Apparently changes in chemical constitution affect the physical properties of these compounds, which in turn determine the rate of diffusion and distribution in the body and the path and rate of excretion of arsenic, thus governing the toxicity for the host and the parasiticidal effects.

Influence of Arsenical Compounds upon Antibody-production in Syphilis. As previously mentioned, one of the oldest theories explaining the curative activity of arsenical compounds supposes that they stimulate the body cells to produce antibodies effecting the destruction of spirochetes. The primary and most important action of these drugs is direct destruction of the parasites, because their action *in vivo* is too rapid for antibody-production in the cure of acute infections with single doses; but since trypan-red and other dyes, as well as organic arsenical compounds, are known to engender temporary immunity following the cure of trypanosomiasis of rats, it is entirely likely that in the treatment of syphilis and other spirochetic infections the repeated administration of the organic arsenical compounds aids in the production of immune substances engendered by antigenic substances from spirochetes after their destruction. As stated in Chapter XVII, my experiments have shown that arsenical and mercurial compounds do not increase antibody-production in rabbits immunized with vaccines of trypanosomes. A similar set of experiments with a vaccine of *Sp. pallida* have yielded like results of a negative character. Rabbits, immunized with *Sp. pallida* vaccine and treated with arsphenamin and neoarsphenamin did not produce agglutinins and alexo-fixagins for *Sp. pallida* any better than the untreated controls.

Insofar as experiments of this kind are concerned, I believe that the results are negative and have shown that arsphenamin and neoarsphenamin, administered to rabbits in amounts analogous to those employed in the treatment of human syphilis, possess no effect upon increasing the production of agglutinins and alexo-fixagins for dead spirochetes.

Naturally, negative results of this kind render improbable that the organic arsenical compounds aid in the production of immunity in syphilis; but are the results directly applicable to syphilis? It is well known that the only resistance or immunity developing in syphilis is due to the presence of living spirochetes in the tissues. Are arsphenamin, neoarsphenamin and other organic arsenical compounds able to aid immunological processes in the presence of living spirochetes, even though they have no influence of this kind with dead spirochetes?

I have no data to offer in answer to this question, because it is difficult to measure resistance or immunity in experimental syphilis and the value of *in vitro* tests is sharply limited. But I believe that, in the treatment of syphilis after each injection of arsphenamin, neoarsphenamin or other active organic arsenical compound, there is a destruction of some spirochetes within the following twenty-four to forty-eight hours by the therapeutically active oxidation or reduction products, followed by the development of some immunity principles engendered by the antigenic substances released from the destroyed spirochetes; and that these immune substances at least aid in holding the activity of spirochetes in check or latency, if they are not actually destructive for them.

Acquired Resistance of Spirochetes to Arsphenamin and Neoarsphenamin.—As stated in Chapter XII, trypanosomes are readily rendered resistant to atoxyl, arsacetin, arsphenamin and other arsenical compounds by treating mice with subcurative amounts or inoculating rats previously treated with the compound. Of far greater practical importance is the question whether or not *Sp. pallida* can be rendered resistant, in the same manner, to arsphenamin, neoarsphenamin and other organic arsenical compounds employed in the treatment of syphilis.

Akatsu and Noguchi[48] were able to increase the *in vitro* resistance of *Sp. pallida*, *Sp. microdentium* and *Sp. refringens* from three to five and one half times their natural resistance to salvarsan and neosalvarsan within a period of 3 to 4 months embracing 6 to 10 transfers. This work was conducted by planting cultures of the spirochetes in 5 c.c. of Noguchi's ascites-kidney-broth medium (not in the agar medium) containing arsphenamin or neoarsphenamin. Preliminary experiments showed that about 0.375 c.c. of a 1:10,000 alkalinized solution of arsphenamin in water added to 5 c.c. of the culture medium would suppress the growth of *Sp. pallida*, but by beginning with smaller amounts and transferring the culture every two weeks to fresh medium containing progressively more arsphenamin, it was found after six to eight transfers that 0.8 c.c. of the solution was not inhibitory. Neosalvarsan was inhibitory at first in dose of 0.5 c.c. of 1:10,000, but after a similar number of such transfers it produced a resistance up to 1.2 c.c. of 1:10,000 solution. Similar resistance was acquired by *Sp. microdentium* and to a lesser extent by *Sp. refringens*. This resistance gradually disappeared when the spirochetes were cultivated in drug-free media for several generations; it is entirely likely, however, that the resistance engendered *in vivo* persists much longer. In acquired resistance of trypanosomes *in vivo* to arsenical compounds, the condition persists for long periods of time.

Klauder[49] has recently shown that resistance of *Sp. pallida* to arsphenamin and neoarsphenamin may be acquired *in vivo* and his results are naturally of great interest and importance in the treatment of syphilis. Klauder found that the minimal curative dose of arsphenamin for rabbits with testicular lesions was about 0.010 gm. per kilogram by intravenous injection. By treating rabbits with subcurative doses and transplanting to fresh rabbits in much the same manner as these tests are conducted with trypanosomes, it was found that after several transfers the resistance acquired was of such degree that the minimal curative dose was more than 0.016 gm. per kilogram of weight. Fantl[50] has reported that the spirochetes disappear less rapidly from lesions of patients who had previously received mercury than from those of untreated cases, and concluded that the

organisms acquired a resistance to arsphenamin as a result of the mercurial treatment. According to his experiments, spirochetes derived from mercury-treated patients (mercuric chlorid and salicylate intramuscularly) remained motile in dark-field preparations in the presence of 0.01 per cent. arsphenamin added to the serum, for only a short time, whereas spirochetes from untreated cases remained motile for hours under the same conditions. Owing to the fact that group resistance of trypanosomes is confined only to compounds bearing chemical relationship, I am inclined to accept these results with much reserve; if mercury does engender a resistance of *Sp. pallida* to arsphenamin, it probably is of but short duration and minor degree.

These experiments show, therefore, that contrary to previous opinion, spirochetes can be rendered resistant to arsphenamin and neoarsphenamin *in vitro* and *in vivo*. My impression is, however, that resistance of *Sp. pallida* is not engenered nearly as rapidly as resistance of the trypanosomes and particularly *Tr. equiperdum*.

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CHAPTER XXIV

TOXICITY AND SPIROCHETICIDAL PROPERTIES OF MERCURIAL COMPOUNDS

A great deal of interest is attached to the possible spirocheticidal activity of compounds of mercury in view of the fact that mercury has been employed for centuries in the treatment of syphilis. An abundance of clinical experience and data shows that mercury possesses curative activity in human syphilis; in frambesia or yaws, however, it has been less efficacious.

In experimental spirochetic infections, mercurial compounds have been found much less curative than the trivalent organic arsenical compounds. One reason for this is the higher toxicity of the former preventing the administration of sufficiently large amounts. *Magna therapie sterilisans* is easily possible in many experimental spirochetic infections with single doses of several of the organic arsenical compounds; it is hardly possible, however, with a single dose of any of the mercurial compounds unless the maximum tolerated or even toxic amounts are given. Aside from this question of organotropism and dosage, mercury is less parasitropic than arsphenamin and other arsenical compounds of this group.

Toxicity and Spirocheticidal Activity of Mercurial Compounds in Relapsing Fever and Fowl Spirillosis.—In *relapsing fever* of rats (*Sp. novyi*), Schamberg, Raiziss and myself[1] found mercuric chlorid, mercury salicylate and benzoate of mercury absolutely ineffective even in amounts as high as the maximum tolerated doses administered by intravenous injection (0.003–0.004 gm. per kilo) within two hours after infection. Of a series of new mercury-naphthol compounds two possessed slight spirocheticidal properties inasmuch as the infection was restrained by them for two to six days. In no instance, however, have we found a mercurial compound capable of effecting complete sterilization.

In experimental *fowl spirillosis*, Uhlenhuth and Manteufel[2] found that a combination of atoxyl and mercury (*mercury arsanilate*) was effective in doses one third to one fifth less than the maximum tolerated amounts. A preparation of colloidal mercury, mercury salicylate and acetylmercury arsanilate were also slightly effective in the maximum tolerated doses, but *Sp. gallinarum* infections are so easily influenced that similar results cannot be obtained in other spirochetic infections. Hahn and Kostenbader[3] found a fluorescin compound of mercury curative in dose of 0.008 gm. per kilogram (maximum tolerated dose 0.018 gm.) and benzolsulphuric acid azomercury salicylic acid slightly curative. Gruhzit[3a] found that *Spirochaeta novyi* in rats is strongly resistant to mercurial medication. The lethal dose of mercuric iodid or acetate was not able to free the animals from spirochetes. Mercurosal when administered before inoculation of rats and followed with daily administrations of three fourths of a minimum lethal dose shortened the life of *Spirochaeta novyi* from five days to three days.

Toxicity and Spirocheticidal Activity of Mercurial Compounds in Experimental Syphilis and Yaws.—In experimental *syphilis* and *yaws*, Nichols[4] found that the intravenous injection of *mercuric chlorid* was practically without effect upon the spirochetes in well developed testicular lesions when given in dose of 0.001 gm. per kilo by intravenous injection. Nichols found this amount to be about the maximum tolerated dose for rabbits although in my experiments the tolerated dose was from 0.002 to 0.003 gm. per kilogram. Nichols found that mercuric chlorid in dose of 0.003 gm. per kilogram of weight had some curative effect in both syphilis and yaws as shown by the disappearance of the spirochetes and healing of the testicular lesions; his rabbits died of nephritis, however, before the final results could be observed. I have had similar results with mercuric chlorid and *mercurophen* and believe that both of these compounds in dose of 0.002 to 0.004 gm. per kilogram (the maximum tolerated doses) can bring about sterilization of the testicular lesions but not usually of the lymphatic glands. Nichols has observed similar results with *mercuric salicylate* (dissolved in a solution of potassium carbonate) and I believe it can be stated that the chemotherapeutic index for these compounds in both rabbit syphilis and yaws is about 1, that is, they are just about curative in the maximum tolerated doses.

Neisser[5] injected 15 monkeys with *mercury salicylate* in dose of 0.052 to 0.120 gm. per animal just before inoculation with virus; 12 developed syphilis and 3 escaped. Of 21 animals injected with 0.050 to 0.080 gm. per animal at the time of inoculation, 15 developed syphilis and 6 did not. Of 13 animals injected with *mercuric chlorid* in dose of 0.030 to 0.035 gm. per animal at the time of inoculation, 9 developed syphilis and 4 escaped. Of 6 animals treated with 19 c.c. of a 0.5-per cent. solution of *colloidal mercury* by intravenous injection, 5 developed syphilis and 1 escaped.

Neisser treated 39 monkeys with *mercury salicylate* after the development of primary lesions; 11 were apparently cured and 22 were not. Of 15 monkeys treated with *mercuric chlorid* after the appearance of primary lesions, 5 were apparently cured and 10 were not. Of 10 monkeys treated with the colloidal mercury preparation, all were apparently cured.

Recently White, Hill, Moore and Young[6] have described a new mercurial compound, the disodium salt of hydroxymercurifluorescein, designated as "flumerin." This compound is a dark red powder with greenish iridescence and is easily soluble in water, 2-per cent. solutions being commonly employed. The compound contains about 32.5 per cent. mercury (theoretical content 33.8 per cent.), with the formula $C_{20}H_{10}O_6NA_2Hg$.

The single maximum tolerated dose for rabbits and dogs by intravenous injection is about 0.030 gm. per kilogram; in dose of 0.005 gm. per kilogram the animals can tolerate repeated doses, even as many as twenty at intervals of three to five days. Inasmuch as the compound contains 32.5 per cent. mercury it is remarkable from the standpoint of low toxicity; I know of no other compound comparable with it from this standpoint. In the treatment of rabbit syphilis single doses of 0.030 gm. per kilogram usually brought about complete sterilization not only of the testicular lesions but of the lymphatic glands as well; multiple doses of 0.005 gm. per kilogram were likewise curative. Flumerin, therefore, is one of the best spirocheticidal mercurial compound known today, but it is to be hoped that additional investigations will be made to evolve a mercurial compound with a higher chemotherapeutic index.

Gruhzit states that *mercurosal* in a single dose of 0.010 to 0.015 gm. per kilogram has cured syphilitic orchitis of rabbits as determined by lymph gland transfer. When administered at two or three day intervals in dose of

0.0033 gm. per kilogram, 70 per cent. of rabbits were cured. Eight doses of 0.0017 gm. per kilogram at intervals of two to five days did not render the lesions free from spirochetes. A dose of from 0.003 to 0.005 gm. per kilogram rendered the lesions free of spirochetes on an average of about three doses and produced no noticeable toxic symptoms in the animals.

Therefore, while mercurial compounds possess some spirocheticidal properties *in vivo*, their high toxicity usually prevents the administration of sufficient amounts to bring about well defined curative effects in single doses; repeated small doses are usually required. From the standpoint of reduced toxicity "flumerin" is an advance, but unfortunately the reduction in toxicity of this compound for the test animal (organotropism) is accompanied by a similar reduction in toxicity for *Sp. pallida* (curative activity or parasitropism) so that the ratio between the two (the chemotherapeutic index) is but slightly better than observed with mercuric chlorid and mercurophen.

Mechanism of Spirocheticidal Activity of Mercurial Compounds.—Practically nothing is known of the mechanism of the spirocheticidal activity of mercurial compounds. They possess an extremely high toxicity for most spirochetes *in vitro*, but the effects *in vivo* are much less powerful. Single doses equal to the sublethal or maximum tolerated amounts are almost without effect in experimental relapsing fever and syphilis although sometimes curative in fowl spirillosis.

It is commonly stated that these results are due to the toxicity of most mercurial compounds which prevents the administration of curative doses. But this is not exactly true. For example, mercuric chlorid can be administered to rabbits by intravenous injection in doses of 0.003 gm. per kilogram giving a dilution in the blood probably not higher than 1:20,000 but this dose has little or no effect upon testicular lesions of syphilis whereas a similar dose of arsphenamin exerts distinctly curative effects.

Furthermore, according to White, Hill, Moore and Young, "flumerin," which contains about 32.5 per cent. of mercury, must be injected intravenously in the maximum tolerated dose of 0.030 gm. per kilogram before curative effects are found in rabbit syphilis. This dose is about three times larger than the minimal curative dose of arsphenamin.

I mention these facts to show that while mercurial compounds are more spirocheticidal *in vitro* than arsphenamin and neoarsphenamin, they are many times less spirocheticidal *in vivo* even when given and tolerated in fairly large doses. That mercury possesses curative activity in human and experimental syphilis cannot be disputed; an abundance of clinical data collected during the past 400 years is ample proof of this fact in human syphilis. But it is highly probable that mercury as administered does not reach or act upon the spirochetes direct, but first requires a process of dissociation with or without the production of new compounds, like union with blood or cellular proteins with the formation of albuminates which are less spirocheticidal than the unchanged compounds. Bergel[7] has recently advanced the theory that mercury is not directly spirocheticidal but acts upon the lymphocytes of the syphilitic exudate releasing from these a lipolytic ferment which is capable of bringing about the gradual destruction of the organisms. While there is little or no direct evidence to support these theories, it is hoped that further researches will yield more information on the exact mechanism of the spirocheticidal activity of not only mercury, but also of bismuth, vanadium, thorium, iodin and other substances as well.

Effect of Mercuric Chlorid upon Antibody-production in Syphilis.—Furthermore there is little direct evidence to support the theory that mercury cures syphilis by increasing the production of antibodies destructive for *Sp. pallida*.

To the best of my knowledge no one has actually demonstrated in a conclusive manner an increase of agglutinins, opsonins or spirocheticidal antibodies in syphilitics attributable to the effects of medicaments including mercurial compounds. It must be admitted, however, that such a possibility exists even though the immunity cannot be measured by *in vitro* tests. We know that certain of the dyes acting on *Sp. novyi* appear to release antigenic substances tending to engender a higher degree of immunity than develops in untreated infections. The phenomenon is even much more apparent in the cure of trypanosomiasis by single doses of trypanocidal dyes, organic arsenical and organic antimonyl compounds. Is a similar phenomenon operative in syphilis treated with mercurial compounds?

My experiments with *Sp. pallida* vaccines have yielded negative results. Rabbits immunized with weekly intravenous injections of a vaccine of culture *Sp. pallida*, accompanied by the injection of 0.0005 gm. of mercuric chlorid per kilogram of weight about 4 hours after each injection of vaccine, have not shown any greater degree of production of agglutinins and alexofixagins (complement-fixing antibodies) than control rabbits receiving the vaccine alone. Several investigators including Toyama and myself, have found that mercury may slightly influence (depress or increase) antibody production for bacteria, but these results cannot be applied to syphilis and the experiments with *Sp. pallida* vaccines have been negative.

But can these results be applied to the effect of mercury upon living spirochetes? As discussed in Chapter XVII the immunity engendered by drugs acting on living trypanosomes is greater than that upon dead trypanosomes. We know that immunity or resistance in syphilis is dependent upon the presence of living spirochetes in the tissues. I have no experimental evidence to show that mercury acting upon living *Sp. pallida* releases antigenic substances capable of engendering a degree of immunity, but I believe such may occur in the treatment of syphilis.

Acquired Resistance of Spirochetes to Mercury.—Is *Sp. pallida* susceptible of acquiring resistance to mercurial compounds? This is a question of fundamental importance in the treatment of syphilis and there is very little data available upon which to express an opinion.

Akatsu and Noguchi[8] have found that the addition of 0.02 c.c. of 1:1000 mercuric chlorid solution to 5 c.c. of ascites-broth effectually inhibited the growth of cultures of *Sp. pallida, Sp. microdentium* and *Sp. refringens*. By starting with smaller amounts and gradually increasing with subcultures every two weeks they were able to raise the resistance of *Sp. pallida* until more than 0.5 c.c. of the 1:1000 bichlorid solution was required within ten weeks, an increase of 25 times the original amount. The resistance of *Sp. microdentium* was raised 10 times and that of *Sp. refringens* 30 times the original dose by this *in vitro* method. This acquired resistance gradually disappeared when the spirochetes were cultivated again in drug-free media for several generations.

Fantl[9] reports that the motility of spirochetes taken from the lesions of persons previously treated with mercuric chlorid or salicylate by intramuscular injection, are more resistant to the effects of mercuric chlorid added to dark field preparations in 0.2 per cent. than the spirochetes from untreated cases.

It is very much hoped that someone will determine if the resistance of *Sp. pallida* to mercurial compounds can be engendered in rabbit syphilis and if so, whether there is a coincident increase of resistance to arsphenamin and neoarsphenamin. I predict, in view of the results observed by Akatsu and Noguchi, that *Sp. pallida* could be rendered resistant in this way but that there would be no, or but slight and temporary, coincident resistance to arsphenamin and neoarsphenamin.

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CHAPTER XXV

TOXICITY AND SPIROCHETICIDAL PROPERTIES OF BISMUTH COMPOUNDS

At the present time bismuth compounds and particularly the tartrobismuthates are commanding considerable attention in the treatment of syphilis and are being especially employed by French syphilologists; indeed, a rather large literature has already accumulated, since Sazerac and Levaditi in 1921 recommended the intramuscular administration of sodium and potassium tartrobismuthate in the treatment of this disease.

The spirocheticidal properties of several organic and inorganic compounds of bismuth, as well as of the metal itself, have been determined in experimental infections of the lower animals which are briefly summarized in this chapter. The curative properties in human syphilis have likewise been extensively reported upon although it is still too early to express a final opinion of the value of bismuth in the treatment of syphilis. In this chapter, I shall briefly review the results of these observations, reserving for Chapter LV a more complete discussion of their toxicity for the lower animals and human beings and for Chapter LXVI, a discussion of their curative properties in the different forms of human syphilis as compared with mercury and the organic arsenical compounds.

Toxicity and Spirocheticidal Properties of Bismuth in Fowl Spirillosis.—The toxicity of bismuth was first investigated experimentally by Balzer[1] in 1899 to determine if it could be used in the treatment of syphilis. Bismuth citrate was chiefly employed but the investigation was abandoned when he found that severe reactions were produced in dogs, especially stomatitis and enteritis. In 1916 Robert and Sauton[2] published the results of a study of the curative activity of sodium tartrobismuthate prepared according to the method of Cowley[3] in experimental fowl spirillosis (*Sp. gallinarum*), which has since stimulated a great deal of laboratory and clinical investigation with bismuth compounds in the treatment of experimental syphilis. These investigators found that *in vitro* a 1:100 solution of sodium tartrobismuthate quickly destroyed the motility of *Sp. gallinarum* in fowl blood.

Toxicity tests showed that the maximum tolerated dose by intravenous injection into normal fowls was 0.030 to 0.035 gm. per kilogram of weight and 0.015 to 0.020 gm. for infected fowls. By intramuscular injection the tolerated dose for healthy animals was found to be 0.100 gm. per kilogram.

When fowls were injected intravenously with a dose of 0.020 to 0.030 gm. per kilogram, or intramuscularly with a dose of 0.050 to 0.070 gm. per kilogram, about four hours before infection with *Sp. gallinarum*, the infection was usually retarded, but complete prevention did not usually occur. Doses of 0.005 to 0.015 gm. per kilogram intravenously and 0.060 to 0.070 gm. intramuscularly, injected six hours after infection, invariably cured the animals. The intravenous injection of 0.015 gm. per kilogram twenty-four hours after infection sometimes proved curative, but seldom or never when given forty-eight and seventy-two hours after infection. Two doses of 0.010 gm. each per kilogram however, generally cured infections of forty-eight and seventy-two hours' duration. They concluded that the tartrobismuthate of sodium, contrary to action *in vitro*, exercises a spirocheticidal action on the *Sp. gallinarum* in the animal. Further work was interrupted at this time by the World War and the death of Sauton.

Toxicity and Spirocheticidal Activity of Bismuth in Rabbit Syphilis.—In 1921, Sazerac and Levaditi[4] and Levaditi and Sazerac[5] conducted extensive experiments with various compounds of bismuth in the prevention and treatment of rabbit spyhilis which have resulted in establishing the value of bismuth in the treatment of human syphilis.

Sazerac and Levaditi infected rabbits with different strains of *Sp. pallida* —their so-called dermotropic and neurotropic strains described in Chapter XXXVI, and after the development of testicular lesions administered the compounds by intramuscular or intravenous injection. As a general rule, the results were evaluated according to the rate of disappearance of spirochetes and healing of the testicular lesions; they do not appear to have removed the lymphatic glands for inoculation to determine if sterilization was complete.

The intramuscular injections of precipitated bismuth (prepared by the reduction of sodium tartrobismuthate), sodium and potassium tartrobismuthate, bismuth oxyiodgallate, bismuthogallate, bismuth lactate and bismuth ammonium lactate, were curative in doses of 0.025 to 0.100 gm. per kilogram of weight, insofar, at least, as to effect a disappearance of spirochetes from the testicular lesions with healing and no evidences of recurrence. The application of a powder of sodium and potassium tartrobismuthate or a 33-per cent. ointment to local lesions possessed prophylactic and curative properties similar to mercury under the same conditions.

Similar results were observed in the treatment of venereal spirochetosis of rabbits, the intramuscular administration of sodium and potassium tartrobismuthate in dose of 0.100 gm. per kilogram of weight, effecting a disappearance of *Sp. cuniculi* and healing of the lesions.

Klauder[6] found that the maximum tolerated dose of sodium and potassium tartrobismuthate in aqueous solution for rabbits by intravenous injection was 0.125 gm. per kilogram. With the oil suspension of the drug, animals survived doses up to 0.2 gm. per kilogram, but succumbed to injections of 0.25 gm. Animals injected with potassium tartrobismuthate survived doses up to 0.25 gm. per kilogram, but died following injections of 0.3 gm. The maximum tolerated dose of bismuth trioxid in olive oil was 0.4 gm. per kilogram. Therapeutic tests were conducted with syphilitic rabbits showing well developed testicular lesions containing numerous actively motile spirochetes. The amounts of the compounds per kilogram of weight required to effect a prompt disappearance of the spirochetes, healing of the lesions and no recurrence during a period of two months was of sodium and potassium tartrobismuthate in aqueous solution and in olive oil and potas-

sium tartrobismuthate in aqueous solution about 0.05 gm. per kilogram of body weight and 0.15 gm. per kilogram for bismuth trioxid.

Kolle[7] has observed that while intravenous injections of mercury and bismuth may not prove spirocheticidal, intramuscular injections are more efficacious in that the development of spirochetes and syphilitic lesions in rabbits may be inhibited. Bismuth deposited in the ears of rabbits for example, was found to prevent the development of scrotal and testicular lesions until the deposited drug was removed by excision, following which the lesions usually developed.

Recently Sazerac and Vaurs[8] have observed that intraperitoneal injections of insoluble bismuth compounds were efficacious in the treatment of *Tr. brucei* and *Spirochaeta cuniculi* infections and they suggest that phagocytes may either change the compounds and render them soluble or else transport the insoluble particles to the infected tissues, although there is an yet no direct evidence of the latter taking place.

Bismuth compounds and especially sodium and potassium tartrobismuthate possess, therefore, well-defined spirocheticidal activity in experimental syphilis. In a general way the single therapeutic dose is about five times smaller than the single maximum tolerated or toxic dose giving a chemotherapeutic index of 5. This index is, therefore, much higher than observed with the mercurial compounds and indeed, higher than observed with all compounds examined to the present time with the exception of the organic arsenical compounds. Milian[9] states that if the chemotherapeutic index of arsphenamin is stated as 10 (that is, the curative dose for rabbit syphilis is 10 times less than the toxic dose), the index for sodium and potassium tartrobismuthate may be stated as 7 and that of mercury at 4. The results of my experiments have been approximately the same for arsphenamin and bismuth but with an index of only 1 for mercurial compounds.

Mechanism of Spirocheticidal Activity of Bismuth Compounds.—Practically nothing is known of the exact mechanism of spirocheticidal activity of bismuth compounds *in vitro* and *in vivo*. In a few experiments, I have found the water soluble potassium and sodium tartrobismuthate spirocheticidal *in vitro* and capable of destroying motility of *Sp. pallida* in high dilutions as well as bringing about their complete destruction as determined by testicular inoculations in rabbits, but in both respects this bismuth compound was less active than mercuric chlorid and mercurophen.

It is highly probable that bismuth acts upon *Sp. pallida* in the tissues in much the same manner as mercury. It is likely that the element forms rather loose combination products with the proteins of the blood and cells which may reduce spirocheticidal activity since parasitropic activity *in vivo* is less than *in vitro*. Following the production of these bismuth albuminates which form *in vitro* much more slowly and less extensively than with mercuric chlorid, the bismuth becomes dissociated and it is highly probable that the bismuth ions are responsible for both the spirocheticidal and toxic effects. Levaditi believes that the bismuth enters a colloidal state and exerts direct spirocheticidal effects in this condition; a further discussion of this subject will be found in Chapter LIV.

Whether or not more complex compounds of essentially different chemical composition are produced *in vivo* similar to the oxidation product of the arsenobenzols and organic antimonyl compounds cannot be stated at the present time. The affinity of bismuth for body cells and parasites is less than exhibited by mercurial compounds but when sufficient amounts are administered the toxic effects upon both have several features in common with the mercurial compounds.

Whether or not bismuth compounds increase antibody production in syphilis cannot be stated at present. It is entirely likely, however, that their effects in this respect are similar to those of mercury. That is, bismuth does not stimulate the antibody producing tissues directly but liberates antigenic principles from spirochetes which hasten the stimulation of these tissues. This process is slow, however, and aids in the treatment and cure of syphilis with repeated doses of a bismuth preparation; in the destruction of spirochetes within the first day or two after administration, the effects of bismuth are direct upon the parasites as this action is too quick for the production of antibodies.

Acquired Resistance of Spirochetes to Bismuth.—It is not known at present whether or not spirochetes and especially *Sp. pallida* acquire increased resistance or fastness to bismuth *in vitro* and *in vivo*, as has been demonstrated with the mercurial compounds *in vitro* and with arsphenamin both *in vitro* and *in vivo*. It is entirely likely, however, that such could be accomplished. Clinical experience has shown that spirochetes resistant to mercury or the arsenobenzols are susceptible to bismuth and for this reason compounds of the latter are useful in the treatment of syphilis when it is suscepted that this resistance or fastness has been acquired in any case or when the body cells have developed an intolerance for arsenic or mercury.

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CHAPTER XXVI

TOXICITY AND SPIROCHETICIDAL PROPERTIES OF THE IODIDS

Owing to the widespread use of potassium and sodium iodids in the treatment of syphilis and yaws, considerable interest is attached to the question of their spirocheticidal activity in experimental infections. Curiously, but little investigation has been devoted to this subject; apparently clinical views to the effect that the iodids in syphilis and yaws are beneficial and curative by aiding the removal of inflammatory tissue without direct destructive effects upon the spirochetes, has been generally accepted.

Toxicity and Spirochaeticidal Activity of the Iodids in Experimental Infections.—In 1910 Tomasczawski[1] stated that he was unable to detect any prophylactic or curative properties of *potassium iodid* in experimental syphilis of rabbits and monkeys, but in 1911 Neisser[2] reported that he was able to prevent and cure syphilis in monkeys by the subcutaneous injection of very large doses of potassium iodid. For example, he injected 7 monkeys with 3.6 to 3.95 gm., corresponding to about 120 gm. for an adult human being, seven

days after inoculation; three animals developed syphilis and four did not. To each of another series of animals he gave 8.25 gm. corresponding to about 210 gm. for the average adult human being, and all escaped infection. A third series received 5.5 gm. each and all developed primary lesions. In other words the dose of potassium iodid required for the prevention of the development of primary lesions in monkeys was from 7 to 8 gm. injected within seven days after inoculation. These doses are too large for administration to human beings, but the experiments show that potassium iodid possesses a slight degree of spirocheticidal activity.

Nichols,[3] in 1911, determined that the maximum tolerated dose of potassium iodid for rabbits by single intravenous injection was about 0.030 gm. per kilo. The intravenous injection of this amount to rabbits with well-developed testicular lesions of syphilis and yaws had no definite curative effects. One rabbit, however, treated with the intramuscular injection of potassium iodid in dose of 1.0 gm. per kilogram of weight, was apparently cured in 10 days; another receiving a subcutaneous injection of 0.5 gm. per kilogram was not benefitted over a period of 10 days and extensive necrosis developed at the site of injection.

Messerschmidt[4] has experimented with fowl spirillosis. Repeated doses of potassium iodid in amounts of 0.1 gm. per kilogram by oral and intramuscular administration had no appreciable preventive or curative effects. One compound designated as *sodium sozoiodid* was definitely curative when given in two doses of 0.1 gm. each per kilogram by intramuscular injection. A number of other iodin compounds as lipojodin, jodipin, jodostarin, jodal, jodoform, jodathylhexamethylentetramin, dioradin, novojodin, sajodin, jodocithin, jodolen, etc., were ineffective in the same dose.

In my experiments the maximum tolerated dose of potassium iodid for rabbits by single intravenous injection was found to be about 0.310 gm. per kilogram of weight; sodium iodid was found very much less toxic being tolerated in doses as high as 2.5 gm. per kilogram (Table 1).

With the assistance of Dr. Malcolm J. Haskins, I have treated a series of rabbits with acute testicular syphilis (about five weeks after inoculation) with daily intravenous injections of potassium iodid in dose of 0.030 gm. per kilo of body weight for 10 days; other rabbits received the drug by stomach tube in dose of 0.1 g. per kilo daily for 10 doses. A third series received sodium iodid intravenously in dose of 0.060 gm. per kilo and a fourth series 0.12 gm. per kilo by stomach tube. These doses were therefore, quite large and corresponded to a daily dose of from 1.8 to 3.6 gm. intravenously and about 6 gm. orally for an adult human being of 60 kilograms. Yet ten consecutive doses of these amounts did not suffice to cure the disease in any rabbit. It is true that the spirochetes were influenced however, because they were reduced in numbers as compared with the untreated controls and in some instances the local testicular lesions healed more rapidly, but when lymph-gland transfers were made to fresh rabbits it was found that with one or two exceptions, living spirochetes were present.

When the iodids are administered to rabbits immediately after inoculation with *Sp. pallida* and the administrations continued at weekly or bi-weekly intervals for a period of four months, the infection is less severe, the lesions less numerous and of shorter duration than in similarly infected but untreated animals, as recently reported by Pearce.[5] In this connection it may be also stated that Akatsu[6] found that potassium iodid was but very feebly spirocheticidal *in vitro*. In his experiments 0.5 c.c. of solutions as strong as 1:10 were required to kill the pallida contained in 3 drops of culture in an exposure

of 1 hour at 37°C. Iodin in the form of Lugol's solution was spirocheticidal in dilution of 1:75.

A general view of these results indicates therefore, that the iodides possess but slight spirocheticidal activity even for *Sp. gallinarum* which is more readily destroyed than *Sp. pallida* and *Sp. pertenuis*. The intravenous injection of potassium iodid in large doses very probably aids in the direct destruction of *Sp. pallida* in the treatment of human syphilis; sodium iodid being 8 times less toxic may be administered in even larger doses for this purpose. But the oral administration of either in the doses commonly employed in the treatment of human syphilis probably has no direct spirocheticidal activity unless this property is enhanced when the drugs are given in combination with either mercury or arsphenamin. This subject is discussed, however, more fully in Chapter XXX and the results of experiments presented.

Mechanism of the Curative Activity of the Iodids in Syphilis.—While the iodids have, therefore, but a slight degree of spirocheticidal activity, an abundance of clinical data shows that the administration of these compounds greatly aids in the reduction and absorption of syphilitic exudates and gummas.

Many different theories have been advanced from time to time to explain the mechanism of this curative activity. Binz[7] and Hinz[8] believed its action to be due to the oxidizing properties of the nascent iodin, which according to Hinz, renders the blood-vessels more permeable and the leukocytes more active. Others have stated that iodin stimulates the activity of the lymphatics with a change in metabolism and an increase of nutritive processes in general promoting the absorption of disease products.

The best explanation has been given by Jobling and Petersen,[9] who believe that the unsaturated fatty acid radicals which act as antiferment and inhibit autolysis become saturated with iodin when the iodids are administered. As soon as this occurs, the ferments which are present in the syphilitic lesions, or which may be brought in, become active, autolysis takes place and the necrotic tissue is absorbed. Here, also, the local action of the ferments is made less difficult by the reduction of the antifrement in the circulating blood. In addition it should be realized that the iodin itself may exert minor degrees of spirocheticidal activity either directly or after the formation of some new compound in the tissues and Pearce is of the opinion that the drug may stimulate or reinforce the host's mechanism of reaction or defense.

Acquired Resistance of Spirochetes to Iodid.—Does *Sp. pallida* acquire an increased resistance to potassium iodid during the treatment of syphilis? This is an important question although since the therapeutic activity of the iodids in syphilis probably does not depend upon spirocheticidal activity to any marked extent, the question is of lesser importance than acquired resistance to the organic arsenical compounds and mercury.

As previously stated iodin is only feebly spirocheticidal *in vitro*. Akatsu and Noguchi found that it required the addition of as much as 0.7 c.c. of Lugol's solution to 5 c.c. of ascites broth medium to inhibit the growth of *Sp. pallida*, *Sp. microdentium* and *Sp. refringens*. By beginning with smaller amounts and gradually increasing with transfers every 2 or 3 weeks, it was found that resistance or tolerance was increased about three fold after 8 to 10 transfers covering a period of 3 to 4 months.

If similar resistance develops *in vivo*, it is probable that during the treatment of syphilis with potassium or sodium iodids that *Sp. pallida* may acquire some resistance to the spirocheticidal activity of these compounds.

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CHAPTER XXVII

TOXICITY AND SPIROCHETICIDAL ACTIVITY OF COMPOUNDS OF QUININ AND OF ANTIMONY, VANADIUM, GOLD, THORIUM AND OTHER COMPOUNDS OF THE HEAVY METALS

Various other substances in addition to compounds of arsenic, mercury and bismuth have been tried in the treatment of spirochetic infections and especially of experimental syphilis of the rabbit. Most interest has been shown in the elements belonging to the same periodic arrangement as mercury and bismuth. Before taking up these other compounds, the spirocheticidal activity of quinin may be briefly discussed.

Spirocheticidal Properties of Quinin.—Since various salts of quinin are well known to be protoplasmic poisons, it is not surprising that quinin was among the substances early investigated in relation to its spirocheticidal properties. Neisser[1] injected 10 monkeys intravenously with *quinin bimuriate* in dose of 0.4 to 0.5 gm. per animal six days after inoculation and all developed syphilis. Two additional animals receiving the same compound by mouth or subcutaneous injection in dose of 3.75 gm. escaped the disease. All of nine monkeys treated in the same way seven days after inoculation developed the disease but of nine more animals treated in the same way with subcutaneous injections 8 days after inoculation, 4 were apparently cured. Of nine monkeys treated after the development of primary lesions with 0.1 to 0.3 gm. of *quinin bimuriate* per animal, 5 were apparently cured. Of 8 additional animals treated with 0.8 to 3.25 gm. by oral administration, 4 were apparently cured.

Schereschewsky[2] has recently reported that quinin salve was effective in preventing natural infection among rabbits by *Sp. cuniculi* (venereal spirochetosis).

It would appear, therefore, that quinin possesses a feeble degree of spirocheticidal activity but these compounds have not been generally employed in the treatment of human syphilis since their curative activity is exceeded by other substances. Quinin is, however, of comparatively low toxicity and the possibilities of improving these compounds for the treatment of spirochetic infections may profitably engage the interests of experimental chemotherapeutists.

Toxicity and Spirocheticidal Properties of Antimonyl Compounds.—As stated in Chapter XIII, antimonyl compounds and especially tartar emetic, possess a high degree of trypanocidal activity. On the basis of this knowl-

edge several investigators have been led to study the spirocheticidal properties of known and new compounds of antimony with the hope that equally good results could be obtained in the treatment of syphilis.

Dubois[3] studied the curative effects of several preparations by intravenous injection in syphilitic rabbits and found that while single doses in amounts equal to the maximum tolerated doses were without much effect, multiple doses of *antimony tartrate* sometimes effected a disappearance of spirochetes from the lesions. When the compound was administered in dose of 0.01 gm. per kilogram along with arsenophenylglycin in dose of 0.01 to 0.03 gm., curative effects were usually observed after two or three treatments. On the basis of these results Dubois thought that antimony may enhance the curative activity of the arsenical compounds and prove worthy in the treatment of human syphilis as a form of combination therapy.

Danysz has combined antimony and silver with arsphenamin in a compound designated as "*luargol*," as a form of combination therapy; the curative dose reported for rabbit syphilis is exceptionally small (0.003 gm. per kilogram) with a chemotherapeutic index of 60. It is probable, however, that while this amount sufficed for the temporary sterilization of the testicular lesions, it is insufficient for complete sterilization of the lymphatic glands.

Uhlenhuth, Mülzer and Hügel[4] and Hügel[5] have prepared a number of new antimonyl compounds which were studied for toxicity and curative activity in fowls infected with *Sp. gallinarum*. Their compounds showed chemotherapeutic indices ranging from 2 to 10, but since fowl spirillosis is so readily influenced it is very doubtful if similar results could be obtained in the treatment of rabbit syphilis, although they report that repeated doses had favorable effects upon *Sp. obermeieri* infections of mice and *Sp. pallida* infections of rabbits.

Hügel[6] observed that the maximum tolerated dose of *potassium antimonyl tartrate* (tartar emetic) for the rabbit was about 0.010 gm. per kilogram by intravenous injection. When this dose was administered to syphilitic rabbits, the lesions showed only non-motile spirochetes the following day but active organisms were again found on the third day. Almost identical results were observed by Klauder[7] with this substance. Klauder also found that *antimony tartrate* in maximum tolerated dose by intravenous injection (0.015 gm. per kilogram) had a similar effect. When administered by intramuscular injection in dose of 0.050 gm. per kilogram, the spirochetes promptly disappeared from the testicular lesions but the animal succumbed in 48 hours.

It may be stated, therefore, that the antimony compounds included in these studies possess but feeble spirocheticidal properties being even less active in this regard than compounds of mercury. However, they are not entirely devoid of spirocheticidal properties and antimony may profitably command further study from the standpoint of producing compounds of lesser toxicity (like sodium antimony thioglycollate of Rountree and Abel) and probably greater spirocheticidal activity.

Toxicity and Spirocheticidal Properties of Vanadium.—Since vanadium belongs to the fifth group of the periodic system of elements in which we find such well-known spirocheticidal substances as compounds of arsenic, bismuth and antimony, Proescher and Seil[8] have studied the toxicity and spirocheticidal activity of a number of its salts. The same paper includes a clinical contribution by Stillians, who found that the salts of vanadium and especially sodium hexavanadate, possess spirocheticidal properties, quickly destroying spirochetes in chancres and mucous patches of human subjects.

This investigation included fourteen compounds of vanadium embracing vanadates, vanadites and vanadyl salts, as well as the more complex derivatives with the oxides of arsenic, antimony and phosphorous. Elaborate chemical, toxicological and various therapeutic studies were made. The toxicity of the vanadium salts appeared to depend on their chemical constitution and was influenced by the number of hydroxyl groups attached to a vanadium atom and the presence of the double bonded oxygen (VO_2 group). Toxicity tests in rabbits by intravenous injection and in guinea pigs, rats and mice by subcutaneous injection, showed that ammonium metavanadate was most toxic (0.0002 gm. per kilogram) and sodium hexavanadate least toxic (0.030–0.040 gm. per kilogram), both values being expressed in terms of vanadium pentoxid (V_2O_5). In experimental animals and human beings, the salts of vanadium may produce nephritis with albumin and casts in the urine as well as gastro-intestinal irritation.

In the treatment of rabbit syphilis Proescher and Seil found that sodium tetravanadate, sodium hexavanadate and vanadyl sulphate injected intravenously in amounts corresponding to about two thirds the maximum tolerated doses, caused a disappearance of spirochetes in scrotal lesions but whether these were curative in the sense of effecting complete sterilization of the lymphatic glands, cannot be stated. Proescher and Seil thought that the spirocheticidal properties were due to the vanadium injected and independent of the salt employed. Negative results were observed in the treatment of rats infected with *Sp. novyi*, but details of these experiments were not given.

Spirocheticidal Activity of Compounds of Gold, Thorium, Germanium, Zinc, Calcium, Tin, Strontium, Cerium, Cadmium, Tungsten, Uranium, Barium and Platinum.—Continuing researches along the same lines, Klauder[9] has studied the spirocheticidal properties of other elementary metals belonging to the fifth group of periodic arrangement and showing possible chemical relationship. This work was conducted by comparing various salts of these elements with the spirocheticidal and symptomatic effects following the intravenous injection of 0.006 gm. arsphenamin per kilogram of weight in rabbits with well-developed syphilitic lesions of the testicles. This dose of arsphenamin is only one half the curative dose but is sufficient for producing primary effects in the testicles and for purposes of comparison without the additional time and extra animals required for determining the minimal amounts necessary for effecting complete sterilization of the lymphatic glands.

Gold chlorid, which has been used in the past in the treatment of locomotor ataxia and other diseases of the central nervous system, was administered intramuscularly in doses of 0.1 to 0.2 gm. per kilogram of weight. Following the 0.1 gm. dose there were no gross changes in the testicular lesion for about five days, but complete involution occurred in sixteen days. Dark-field examination twenty-four hours after treatment showed many non-motile spirochetes (before treatment many very motile spirochetes were present), but an absence of spirochetes was not noted until the sixth day after treatment. Since a similar result is within the limit of the rate of disappearance of the syphiloma and of spirochetes in untreated animals, this amount of gold chlorid was not regarded as exerting spirocheticidal action.

Following treatment with 0.150 gm. of gold chlorid, the syphiloma involuted in twelve days; the dark-field examination was negative in three days. This amount, therefore, furnished evidence of some spirocheticidal activity. Treatment with 0.200 gm. killed the animal in five days but until this time there was little evidence of spirocheticidal activity of the drug. It is apparent, therefore, that maximum doses of gold chlorid do not possess the spirocheticidal activity of 0.006 gm. arsphenamin per kilogram, but nevertheless gold is not entirely lacking in this property. Insofar as effect upon rabbit syphilis is concerned, gold chlorid in single maximum tolerated doses exerts almost as much spirocheticidal action as the salts of mercury.

Thorium chlorid demonstrated some spirocheticidal activity. The intramuscular injection of 0.100 gm. per kilo produced a partial involution of the syphiloma during the following ten days; the dark-field examination became negative the sixth day after treatment, but showed spirochetes again in fifteen days, at which time the syphiloma was well pronounced. Treatment with 0.125 gm. per kilogram produced a marked improvement of the syphiloma in ten days and involution in seventeen days; the dark-field examination became negative in six days and there was no recurrence over a period of observation of sixty days. Two animals were treated with 0.150 gm. of thorium chlorid per kilogram. In one there occurred an involution of the syphiloma in nine days and the dark field examination became negative in two days. The second showed a marked improvement of the syphiloma two days following treatment, but complete involution did not occur until two weeks; the dark-field examination became negative in six days.

Thorium chlorid, therefore, is not entirely devoid of spirocheticidal properties and in single maximum tolerated doses exerts a curative effect in rabbit syphilis analogous to that of vanadium, gold and even mercury.

All other salts of these elements including *germanium*, *zinc*, *calcium*, *tin*, *strontium*, *cadmium*, *tungsten*, *uranium*, *barium* and *platinum*, yielded negative results when administered in single doses of 0.100 to 0.125 gm. per kilogram (germanium 0.005 to 0.025 gm.) by intramuscular injection. Dark-field examinations of the testicular lesions made one, two and more days after treatment showed no influence upon numbers and motility of spirochetes. Most interest was commanded by germanium oxide on account of its close relationship to arsenic, but this substance possesses neither trypanocidal nor spirocheticidal properties in single maximum tolerated doses. Strontium and platinum have been com-

bined with arsphenamin and these compounds possess well marked spirocheticidal properties, but the simple chlorides of the metals themselves are devoid of spirocheticidal activity.

Neisser[10] has studied the prophylactic and curative properties of various colloidal preparations of the heavy metals in syphilis of monkeys. Of eight animals treated with 0.1 to 0.25 gm. of *collargol* by intravenous injection of 1-per cent. solutions after the development of primary lesions, 4 were apparently cured and 4 were not. Eighteen monkeys treated with 13–30 c.c. of *elektrargol* were not cured. Six monkeys treated with 16–20 c.c. of *elektropalladiol* were not cured but two additional monkeys treated 5 days after inoculation, escaped syphilis. Of ten monkeys treated with *elektroplatinol* 5 to 6 days after inoculation, 9 developed syphilis. All of 7 animals treated with 15 c.c. of *elektraurol* immediately after inoculation developed the disease; likewise all of 8 additional monkeys treated with *cuprol* by oral administration (3.75 gm. in 30 days). Six monkeys treated with *cuprargol* by oral administration (3.75 gm. in 3 weeks) were not cured.

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CHAPTER XXVIII

SPIROCHETICIDAL PROPERTIES OF ARSPHENAMIN, NEOARSPHENAMIN, MERCURIAL AND OTHER COMPOUNDS IN VITRO

The results of test tube or *in vitro* spirocheticidal tests are greatly influenced by the technic employed. It is not possible to compare the results observed by one investigator with the results of others unless the same method has been used. For this reason the results of spirocheticidal tests *in vitro* should be evaluated only in a broad and general manner.

These tests, however, are not without some practical significance. As discussed in Chapter IV, *in vitro* bacteriostatic and bactericidal tests have a definite value in chemotherapeutic research and the same may be said of spirocheticidal tests, since we now have available methods for cultivating various pathogenic species. Furthermore, they have some application to the treatment of local infections like Vincent's angina and spirochetic gingivitis by the local application of spirocheticidal substances; likewise to the prophylaxis of syphilis, as shown by the investigations of Reasoner[1] and others discussed in more detail in the chapter devoted to the prophylaxis of syphilis.

Hata found that a 1:4000 solution of salvarsan destroyed the motility of *Sp. obermeieri* in the test tube and actually killed the organisms as determined by injecting a portion of the mixture into mice. However, controls of 1:4000 solutions containing the same amount of sodium hydroxid as in the salvarsan solution yielded the same results.

Castelli[2] found that in mixtures of 1 c.c. of varying dilutions of neosalvarsan up to 1:1000 in saline solution and 1 c.c. of chicken blood containing *Sp. gallinarum* kept at room temperature for one hour followed by the injection of 0.3 c.c. of the mixtures into fowls, there was no evidence of an actual destruction of these spirochetes. Similar experiments with *Sp. obermeieri* showed that while neosalvarsan was unable to kill the organisms in dilutions up to 1:1000, the virulence or infectivity of the spirochetes was reduced for

mice. Hahn and Kostenbader[3] reported that a fluorescin mercury compound capable of curing *Sp. gallinarum* infections of fowls in dose of 0.008 gm. per kilogram (maximum tolerated dose 0.018 gm.) was without effect *in vitro* when mixed with blood containing the spirochetes for 15 to 45 minutes followed by intramuscular injection into chickens to determine spirocheticidal effects.

On the basis of these experiments, both Ehrlich and Hata taught that salvarsan possessed no spirocheticidal activity *in vitro*. This view is still maintained by many physicians, although it has since been shown by Neufeld and Böcker[4] that salvarsan and neosalvarsan are able to kill *Sp. gallinarum* in the test-tube and that both substances possess spirocheticidal properties *in vitro* for suspensions of *Sp. pallida*.

Noguchi and Bronfenfrenner,[5] for example, mixed 0.1 c.c. of culture with 0.5 c.c. of varying dilutions of salvarsan and after one hour at room temperature cultured the mixture in the ascites kidney agar medium of Noguchi. Controls were included of the sodium hydroxid alone. Salvarsan was found to kill *Sp. pallida* under these conditions in dilutions as high as 1:1000; similar results were observed with cultures of *Sp. refringens*, *Sp. mucosum* and *Sp. microdentium*. Sodium hydroxid in dose of 0.5 c.c. of $\frac{N}{10}$ to $\frac{N}{30}$ strength killed 0.1 c.c. of the various cultures in the exposure of one hour.

Noguchi and Bronfenbrenner also found that arsenious acid possessed a high degree of spirocheticidal activity *in vitro* although this compound does not influence spirochetic infections of the lower animals even in maximum tolerated doses. Mercuric chlorid was spirocheticidal in dilutions as high as 1:200,000 and tricresol and phenol in dilutions of 1:200 to 1:500. In comparative tests with a culture of *B. coli*, it was found that all of these substances were from 20 to 100 times more toxic for spirochetes than for the bacillus.

Noguchi and Bronfenbrenner also made the interesting observation that the spirocheticidal activity of salvarsan was increased two to five times in the presence of defibrinated rabbit blood and fresh emulsions of rabbit liver. For example, a 1:1000 solution of salvarsan in saline solution was spirocheticidal but a 1:2000 dilution in a fresh unheated extract of liver and a 1:5000 dilution in rabbit blood were equally spirocheticidal. Since a solution in a boiled extract of liver was spirocheticidal only in 1:700, Noguchi and Bronfenbrenner thought that the liver enzymes increased spirocheticidal activity, a view somewhat similar to that expressed by Levaditi. These results are not without significance in view of the fact that arsenic is stored in the liver after the administration of arsphenamin and neoarsphenamin; furthermore, the liver is more subject to the toxic action of these compounds than any other organ, as discussed in Chapter XLVI.

Schamberg, Raiziss and myself[6] determined the spirocheticidal activity of salvarsan and mercuric chlorid by mixing 1 c.c. of varying dilutions of each with 1 c.c. of *Spirochæta pallida* culture (Zinsser's strain A) and after two hours in the water bath at 37°C., 10 c.c. of ascites-kidney agar and a piece of sterile kidney were added under anerobic conditions for several weeks and examined after the controls showed good growths. Under these conditions it is possible that the compounds continued to exert some spirocheticidal activity throughout the period of incubation. Different lots of arsphenamin were spirocheticidal under these conditions in dilutions varying from 1:2000 to 1:128,000; mercuric chlorid was spirocheticidal in dilution of 1:160.

In a later study by Akatsu[7] in Noguchi's laboratory, additional evidence was produced showing that salvarsan and neosalvarsan possessed spirocheticidal activity *in vitro*. Akatsu prepared solutions of these and other compounds in distilled water and to 1 c.c. of each dilution added three drops of culture. After standing one hour in a water bath at 37°C. transplants were made to Noguchi's ascites agar medium and the cultures incubated anerobically for several weeks. With this method Akatsu found that salvarsan was spirocheticidal in dilutions as high as 1:7500. Neosalvarsan was less toxic for *Sp. pallida*, since the highest spirocheticidal solution was 1:2500. Mercuric chlorid had a value of 1:100,000; phenol, 1:2500; lysol, 1:500; formalin, 1:750, etc. Sodium cholate, sodium glycocholate and taurocholate and sodium oleate were likewise highly toxic proving spirocheticidal in dilution of 1:2500 to 1:7500 and confirming the observations of Neufeld and von Prowazek[8] and Gonder[9] that spirochetes, unlike bacteria in general, are highly susceptible to the lytic action of sodium taurocholate and saponin. Akatsu also found that 76 of the new quarternary salts of hexamethylene tetramin compounds prepared by Jacobs and Heidelberger,[10] possessed spirocheticidal properties in dilutions of 1:50 to 1:50,000.

Lee[11] has studied the spirocheticidal activity of salvarsan and neosalvarsan by mixing a drop of varying dilutions on slides with a drop of a suspension of *Sp. pallida* obtained from well developed chancres or mucous patches of human subjects. By sealing with vaseline to prevent evaporation it was possible to study the motility and morphology of the spirochetes with the dark field illuminator for 8 hours or longer. With this method Lee found that 1:130 solutions of arsphenamin, neoarsphenamin and silver arsphenamin were without effect upon the spirochetes; likewise arsphenaminized and neoarsphenaminized serum from blood drawn two to forty-eight hours after the intravenous injection of these com-

pounds. On the other hand an emulsion of tissue removed from a syphilitic lesion showing a reaction of hyperemia after the injection of salvarsan was highly spirocheticidal. On the basis of these results, Lee believes that salvarsan exerts its spirocheticidal properties by first combining with the protein of cells forming an "arsenoprotein" detrimental to *Sp. pallida*, whereas the mercury salts are direct chemical poisons.

Greenbaum, working in my laboratory, employed a similar method for determining the toxicity of arsphenamin, neoarsphenamin, arsenious acid, mercurophen, mercuric chlorid and other substances for the numerous kinds of spirochetes in the secretions of the gums of human subjects with spirochetic gingivitis. Slides were prepared by mixing one loopful of gingival spirochete emulsion with one loopful of solution of drug followed by sealing and study with the dark field illuminator at frequent intervals up to 2 hours. The following results were observed, the dilutions indicating the highest required to effect complete loss of motility of the spirochetes with morphological changes: Arsphenamin, 1:200 instantaneous; 1:500 required 1 to 2 hours. Neoarsphenamin 1:50 instantaneous; 1:100 required 1 to 2 hours. Mercurophen and mercuric chlorid 1:6000 instantaneous. Arsenious acid, and iodin 1:50 had no effect. These results show that arsphenamin, neoarsphenamin and mercurical compounds are able to exert a toxic effect upon spirochetes in the presence of inflammatory exudates and I shall refer again to these studies in a later chapter dealing with the local treatment of spirochetic gingivitis with solutions of arsphenamin.

Weiss and Wilkes working in my laboratory, have brought forward additional evidence showing the spirocheticidal activity of arsphenamin, neoarsphenamin and other substances *in vitro*. They have prepared isotonic solutions of each substance in varying strengths and mixed 0.5 c.c. of each in test tubes with 0.5 c.c. of *Sp. pallida* cultures. These mixtures were placed in a water bath at 37°C. and at varying intervals each mixture was examined for the motility of spirochetes and cultured anerobically in gelatinized horse serum for the viability of the organisms. The pH of each solution of arsphenamin and neoarsphenamin was determined and control solutions of isotonic saline prepared with the same pH reaction. In this way the influence of alkali upon the spirochetes was adequately checked.

Both arsphenamin and neoarsphenamin brought about a loss of motility and death of the spirochetes in dilutions of 1:200 to 1:1000, arsphenamin being the more toxic. Since the control solutions in 1:200 were not toxic the results prove conclusively that both compounds are spirocheticidal *in vitro*. The spirocheticidal activity of copper sulphate, halozone and mercurophen was also shown. Brilliant green, however, was not spirocheticidal in dilution of 1:1000 although this strength was highly bactericidal.

Finally, I have been able to show the well defined spirocheticidal activity of arsphenamin, neoarsphenamin and mercuric chlorid *in vitro* by mixing 0.5 c.c. of varying isotonic dilutions in test tubes with 0.5 c.c. of an emulsion of virulent *Sp. pallida* secured by emulsifying rabbit syphilitic testicles containing numerous actively motile spirochetes. These mixtures were kept in a water bath for 15 minutes, examined for motility and 0.5 c.c. of each injected into the testicles of rabbits. The animals were then kept under observation for several months. A dilution of 1:400 of arsphenamin destroyed motility and 1:1600 was spirocheticidal. Neoarsphenamin in dilution of 1:200 reduced motility and 1:1600 was spirocheticidal, being less toxic for the organisms than arsphenamin in both tests. Mercuric chlorid destroyed motility in 1:10,000 and was spirocheticidal in at least 1:20,000 being at least several times more spirocheticidal *in vitro* than either arsphenamin or neoarsphenamin. Gruhzit[12] has observed that mercurosal is less spirocheticidal *in vitro* for *Spirochaeta novyi* and *Spirochaeta pallida* than the inorganic mercurials; the latter appeared to be from five to seven times less resistant than *Spirochaeta novyi*. For example, 1:25,000 mercurosal destroyed the motility of *Spirochaeta pallida* in one hour but *Spirochaeta novyi* required 1:10,000; the motility of both spirochaetes however, was instantly destroyed by mercuric chlorid and iodid of mercury in these dilutions.

A general review of the results recorded shows, therefore, that both arsphenamin and neoarsphenamin possess spirocheticidal properties *in vitro* for various spirochetes. However, these toxic effects are apparently far more evident with cultures of spirochetes than with spirochetes in inflammatory exudates. Whether or not the curative action of the organic arsenicals in the living animal can be explained on the basis of direct spirocheticidal action of arsphenamin and neoarsphenamin as such or in the form of their oxides, is still an open question. The mercurials are especially toxic for spirochetes *in vitro* and if their effect *in vivo* were a simple direct one it would be expected that they would show more curative effects. Even though 0.002–0.003 gm. of mercuric chlorid per kilogram is the maximum tolerated dose, yet the intravenous injection of these amounts has no effect upon trypanoso-

miasis or rabbit syphilis while a similar dose of arsphenamin brings about a partial sterilization and even a cure in some instances. Since mercuric chlorid is more than 10 times more spirocheticidal than arsphenamin *in vitro*, it is apparent that the mechanism of spirocheticidal activity *in vivo*, which reduces the parasitropism of mercury and increases that of arsphenamin and neoarsphenamin, is a far more complicated process than direct chemical toxicity of these substances for various spirochetes.

Since *bismuth* has become widely employed in the treatment of syphilis, considerable interest is attached to the effects of its soluble salts upon *Spirochaeto pallida in vitro*. With the assistance of Miss Anna Rule, I have studied the influence of potassium tartrobismuthate and sodium tartrobismuthate upon spirochetes derived from acute testicular lesions of rabbits; final dilutions as concentrated as 1:20 were required to produce loss of motility of all spirochetes in 5 minutes at 38°C.; with an exposure of 30 minutes at this temperature loss of motility occurred in final dilutions of about 1:80. Saline controls kept at 37°C. showed loss of motility in 60 minutes but controls kept at room temperature showed no loss of motility in 2 hours, indicating that saline solution was much more toxic for these spirochetes at 37°C. than at approximately 20°C.

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CHAPTER XXIX

METHODS OF ADMINISTRATION IN RELATION TO THE CHEMOTHERAPY OF EXPERIMENTAL SYPHILIS

In the preceding chapters the minimal curative doses of various arsenical, mercurial bismuth and other compounds for syphilitic infections of rabbits, have been generally expressed according to the smallest amounts per kilo of weight capable of dissipating the local lesions and sterilizing the lymph-glands. These amounts are summarized in Chapter LXVII but are too large for administration to human beings when given according to body weight, because of toxic effects. The hope of *magna therapie sterilisans* in the treatment of human syphilis has been abandoned, at least for the present time, and multiple doses are given which renders important the subject of intervals between administrations. Under ordinary circumstances syphilis of the rabbit is much milder than syphilis of the human being, although suppression of the primary lesion sometimes results in the widespread distribution of

spirochetes with the production of lesions of the skin and mucous membranes, lymph glands, bones, special organs, etc., similar to the lesions of syphilis in the human being. But natural immunity in the rabbit to *Spirochaeta pallida* is more marked than in human beings; likewise, the immunity response in untreated and treated syphilis of this animal is more decided and these factors render the cure of syphilis of the rabbit much easier of accomplishment than syphilis of human beings; however, data of much value may be obtained from well conducted experiments employing these animals.

Importance of Intervals between Administration of Medicaments in the Treatment of Acute Syphilis of Rabbits.—It is difficult to express the comparative curative values of antisyphilitic medicaments in divided doses, because of the marked variation in results with rabbits and the very large number of tests required in order to obtain general and average results. However, with a few of the antisyphilitic remedies fairly accurate figures have been obtained and these have indicated that *when the treatments are given once a week, the total amounts required for effecting a complete cure in the primary stage of syphilis are greater than when the medicaments are given at shorter intervals.* Apparently in the longer intervals of administration of medicaments in amounts less than the single curative dose, surviving spirochetes are enabled to proliferate, acquire some degree of fastness or by some other agency require a higher *total* amount of the compound to effect complete sterilization than is the case when the medicament is given in a single large dose or in smaller amounts at daily or two day intervals. These results are shown in the following table:

Compound	Total Required per Kilo in One Dose (Gm.)	Total Required per Kilo in 3 Doses Given at Daily Intervals (Gm.)	Total Required per Kilo in 4 Doses Given Twice Weekly (Gm.)	Total Required per Kilo Given in 6 Doses Once a Week (Gm.)
Arsphenamin	0.012	0.015	0.024	0.048
Neoarsphenamin	0.020	0.024	0.040	0.090
Sod. and potass. tartro-bismuthate	0 050	0.060	0.060	0.090

In the treatment of acute primary testicular syphilis of the rabbit, *the intervals between the administration of medicaments is, therefore, a matter of considerable importance* and the same has been found true in the treatment of trypanosomiasis of these animals with the pentavalent and trivalent organic arsenical compounds; indeed, proper spacing of the injections is almost as important as the size and number of doses.

Comparative Study of Methods for the Administration of Arsphenamin, Neoarsphenamin, and Bismuth in the Treatment of Chronic Syphilis of Rabbits.—In the treatment of chronic syphilis of the rabbit however different results have been observed. By inoculating large numbers of rabbits and castrating seven to fourteen days later to promote dissemination of the infection, a sufficient number were suitable for chemotherapeutic studies from six to eight weeks later. In the majority of instances the animals showed some loss in weight with lymphatic, cutaneous, muco-cutaneous and periosteal lesions. The Wassermann reactions, conducted according to my new method, showed fixation with 0.025 c.c. and smaller amounts of serum; amounts greater than 0.025 c.c. were not employed, in order to avoid the non-specific reactions sometimes encountered with the sera of normal rabbits.

The criteria of cure in these experiments, conducted with the assistance of Miss Anna Rule, were disappearance of the lesions, a reduction of the complement-fixation reactions and *negative rabbit inoculation tests with emulsions of lymph-glands.* The latter was regarded as the best evidence, because, as shown by Brown and Pearce and amply confirmed by us, living *Sp. pallida* may be found in the inguinal and popliteal lymph-glands in cases of extreme latency and apparent cures. The medicaments selected were arsphenamin (disodium salt), neoarsphenamin and sodium and potassium tartro-bismuthate, all except the bismuth being administered by intravenous injection in order to render dosage and absorption as accurate as possible. The total amount required for effecting cures of the majority (not all) of the rabbits were approximately as summarized in the following table:

Compound	Total Required per Kilo in One Dose (Gm.)	Total Required per Kilo in 6 Doses Given Twice Weekly (Gm.)	Total Required per Kilo in 6 Doses Given Once a Week (Gm.)
Arsphenamin	0.030	0.090 to 0.120	0.080 to 0.100
Neoarsphenamin	0.050	0.120 to 0.250	0.110 to 0.240
Bismuth	0.090	0.06 to 0.180	0.06 to 0.160

It will be noted that the single curative doses were two to three times larger than required for the cure of acute testicular syphilis and in the case of bismuth, about one-half the maximum tolerated dose, even though this amount was not always curative. The single curative doses, while less than the *total* amounts required when injected once or twice weekly, are altogether too large and out of question in relation to the treatment of chronic syphilis of human beings; therefore it is interesting to note that the *total* amounts of the various medicaments required when given once a week were slightly less than when given twice a week. Furthermore, fewer animals were lost by the once a week plan. These experiments have also shown that in the treatment of the cutaneous, muco-cutaneous and periosteal lesions of rabbits, better results were secured with arsphenamin and neoarsphenamin than with bismuth and mercury. These lesions in the rabbit can be interpreted as chronic lesions of mild degree, even though they developed and were usually treated within a period of four months after inoculation. The trivalent arsenical compounds, at least, were apparently more penetrative and curative than the bismuth preparation in these experiments and were less toxic in divided doses at intervals of administration of about three days.

Comparative Study of Methods for the Administration of Mercury in the Treatment of Chronic Syphilis of Rabbits.—In Chapter LII I have discussed the influence of route of administration of mercurial compounds upon toxicity; here may be recorded a summary of experiments bearing upon the influence of route of administration upon curative activity in chronic syphilis of the rabbit. In conducting these experiments the following three mercurial compounds were chosen for intravenous, intramuscular and cutaneous administration in amounts per kilogram of body weight, corresponding closely to the amounts commonly employed in the treatment of syphilis of human subjects based upon 130 pounds of weight. (a) *Mercuric chlorid* by intravenous injection in dose of 0.00025 gm. per kilo (corresponds to ¼ grain per 130 pounds), twice a week. (b) *Mercuric chlorid* by intramuscular injection in same dose once per week. (c) *Salicylate of mercury* by intramuscular injection in dose of 0.001 gm. per kilo (corresponds to 1 grain per 130 pounds), once a week. (d) *Mercurial ointment* (U.S.P.) by inunction in dose of 0.06 gm. per kilo (corresponds to one drahm per 130 pounds), once a day. For administration the ointment was diluted with benzoated lard and lanolin to permit the application of larger bulk and consequently with less opportunity for error. Since the ointment contained 33 per cent. of metallic mercury, the dose of 0.06 gm. per kilogram corresponded to about 0.02 gm. of mercury.

The results summarized in the following table shows the approximate number of doses required for effecting cures in at least 75 per cent. of each of the three groups of animals:

Compounds	Per Cent. Mercury	Dose per Kilo (Gm.)	Route of Administration	Not Curative	Curative	Total Amount Sometimes Curative (Gm. per Kilo)
Mercuric chlorid	74	0.00025	Intraven.	12 doses	30 doses	0.0075
Mercuric chlorid	74	0.00025	Intramus.	10 doses	42 doses	0.0105
Salicylate mercury	45	0.001	Intramus.	15 doses	38 doses	0.038
Mercurial ointment	33	0.06	Cutaneous	24 doses	45 doses	2.7

It was not possible to obtain more accurate data without greatly increasing the labor and expense of the study, because of the large number of rabbits required for furnishing a suitable number of cases presenting approximately similar lesions for the four different forms of treatment. But the results are regarded as approximately correct and indicate the following:

(a) Best results were observed with the intravenous injection of a soluble salt of mercury (mercuric chlorid). The lesions and Wassermann reaction were dissipated more promptly and the total amount of mercury required for cures was less than with other routes of administration.

(b) Next best results were observed, in the order named, with intramuscular injections of a soluble mercurial compound (mercuric chlorid), an insoluble compound by intramuscular injection (salicylate) and metallic mercury by inunction (blue ointment).

(c) Insofar as mercuric chlorid and the salicylate are concerned, it would appear that the compounds are equally curative when judged upon the basis of actual amounts of metallic mercury administered. Mercury salicylate cures more slowly, owing to slower absorption of mercury, but the differences in curative activity of mercuric chlorid, the salicylate and the ointment depend upon different rates of absorption of mercury. After absorption has occurred, the mercury in each is probably of equal curative activity, just as the toxicity of these compounds appears to bear a relation to the actual amounts of mercury absorbed irrespective of the kind of compound or route of administration. A combination of these compounds, as arsphenamin or neoarsphenamin with a mercurial, yielded somewhat better results in treatment than a single compound and especially when injected intravenously. This subject is discussed in the following chapter: likewise, the curative activity of potassium iodid in chronic syphilis of the rabbit when administered with an arsenical or mercurial compound.

Intravenous versus Intramuscular Injection of Antisyphilitic Drugs.—Within recent years there has been an increasing tendency to administer antisyphilitic drugs intravenously in the treatment of human syphilis whenever conditions permitted this route of administration. When arsphenamin was first introduced by Ehrlich, intramuscular injection was employed, but it was soon found that this procedure produced great pain and tissue necrosis at the site of injection. To overcome this difficulty, the intravenous injection of the alkalinized solution (disodium salt) was introduced, and this method subsequently met with universal adoption. It was hoped that neoarsphenamin would overcome these difficulties of intramuscular injection, but it was soon found that even this compound causes considerable local reaction, although the degree of irritation may be diminished by the use of an oil suspension instead of a water solution. Still more recently, sulfarsenol or sulpharsphenamin have been introduced for subcutaneous and intramuscular injection, and these produce even less local irritation than neoarsphenamin in the majority of persons.

Many other factors require consideration in addition to this important one of local reaction in relation to the route of administration, not only of the arsenobenzenes but of mercury and bismuth as well, in the treatment of syphilis. For example, the rapidity and degree of absorption and elimination are of considerable importance as discussed in Chapter XLIII as well as general toxic effects and therapeutic activity. In general terms it may be stated that while intravenous injections may be painless, the chances of producing general toxic reactions are greater with all antisyphilitic drugs than by intramuscular injection. It is apparent, however, that the subject cannot be dismissed with a few brief and superficial statements and at this time I shall discuss the subject only from the standpoint of comparative curative activity in experimental infections of the lower animals, leaving for subsequent chapters a discussion of the subject from the standpoint of local and general toxic reactions, absorption and elimination and practical applications in the treatment of human syphilis.

Rabbits inoculated intratesticularly and treated after the development of large acute lesions with numerous actively motile spirochetes, have shown the following *minimal curative doses per kilo-gram of weight*, these amounts being approximately the smallest doses required not only for the dissipation of the testicular lesions and spirochetes but sterilization of the lymphatic glands as well:

Arsphenamin (disodium salt) intravenously	0.012 gm.
Arsphenamin (olive oil) intramuscularly	0.015 gm.
Neoarsphenamin (water) intravenously	0.015 gm.
Neoarsphenamin (olive oil) intramuscularly	0.015 gm.
Sulpharsphenamin intravenously	0.022
Sulpharsphenamin (water) intramuscularly	0.016

Owing to the feeble spirocheticidal activities of mercury and bismuth compounds, comparative tests of this kind have not yielded as definite information as the arsenobezene compounds. Experiments with rabbit syphilis, however, have shown that repeated intramuscular injections of soluble mercurial compounds, as mercurophen and flumerin, have yielded practically the same results as repeated intravenous injections of the same compounds in the same doses per kilogram of weight; it is highly probable that the same holds true for other soluble salts employed for the treatment of syphilis, although the curative activity of the insoluble salts as calomel and the salicylate emulsified in oil, is slightly less curative than the soluble salts administered by intravenous injection.

Intramuscular injections of solutions of bismuth salts (sodium tartro-bismuthate, potassium tartro-bismuthate, sodium and potassium tartro-bismuthate) in water have yielded approximately the same single minimal curative doses per kilogram of weight in rabbit syphilis as intravenous injections of these compounds. Suspensions of these salts in sterile olive oil injected intramuscularly have been, however, slightly less curative than intravenous injections of the compounds dissolved in water. Unfortunately, the bismuth salts are quite toxic by intravenous injection and solutions in water produce rather severe local reactions upon intramuscular injection, but the curative activity of intramuscular injections of emulsions in olive oil is only slightly less than intravenous and intramuscular injections of these salts dissolved in sterile water.

Experiments have shown, therefore, that intramuscular injections of the arsenobenzenes, mercury and bismuth compounds possess almost, if not more, curative activity as intravenous injections in the treatment of syphilis of rabbits when the same doses are administered per kilogram of weight. Of course, the effects of intramuscular injections are slower and especially after the injection of emulsions in olive oil, which reduces the value of intramuscular injections in the treatment of acute infections, but the ultimate effects of intramuscular injections of soluble antisyphilitic compounds are approximately the same as observed after the intravenous injection of these. The main advantages of intramuscular injections are greater freedom from general toxic reactions, slower absorption, slower elimination and thereby a longer range of therapeutic activity; the chief disadvantages are local pain, necrosis and disability when adequate doses are administered. The discovery of an organic arsenical compound possessing the same or higher therapeutic efficiency as arsphenamin and neoarsphenamin, but which may be administered intramuscularly or subcutaneously *in adequate and repeated doses*, will prove extremely useful in the treatment of human syphilis; at the present time sulpharsphenamin is best adapted for intramuscular or subcutaneous injection and is well borne by most individuals.

CHAPTER XXX

COMBINATION CHEMOTHERAPY OF EXPERIMENTAL SYPHILIS AND ITS RELATION TO THE TREATMENT OF HUMAN SYPHILIS

The subject of the combination therapy of syphilis is deservedly worthy of considerable attention since it would appear possible to increase the spiro-

cheticidal activities of two different compounds administered together beyond the summation of the effects of both separately without an increase of toxic effects. I have already discussed the subject in relation to the chemotherapy of experimental trypanosomiasis in Chapter XIX, stating that mixtures of arsphenamin and neoarsphenamin with certain mercurials and other compounds have sometimes increased trypanocidal effects beyond what could be expected from the compounds separately.

Kolle[1] believes that some mercurial compounds administered simultaneously with arsphenamin or neoarsphenamin hastens the transformation of the latter into therapeutically active substances without an increased toxicity. Bergel[2] also states that combined treatment yields best results, but believes that the mercury releases from the lymphocytes a lipolytic ferment which is spirocheticidal and thereby supplementing the curative effects of arsphenamin or neoarphenamin.

Apparently best results have followed when solutions of the arsenical and mercurial compounds were mixed and injected together, as in Linsser's method of combination therapy of human syphilis. As stated in Chapter XXIV, attempts toward evolving compounds of organic arsenic and mercury have so far failed to produce substances of distinctive curative properties although more recent attempts in combining arsenic and bismuth are apparently more successful. Kolle states that mixtures of salvarsan and mercuric chlorid or neosilversalvarsan and novasural are not the same as the metal (silver, gold, platinum, etc.) salvarsans, because the mercury does not enter into chemical union with the arsenic molecule; colloidal mixtures only are produced which tend to precipitate (Tyndall's phenomen) and especially mixtures of bichlorid and arsphenamin. Mixtures of novasurol and neosalvarsan remain clear and I observed similar results with mixtures of mercurophen and flumerin with arsphenamin or neoarsphenamin.

Kolle has found that the single minimum curative dose of neosilversalvarsan for scrotal chancres and testicular lesions of rabbits was about 0.010 to 0.015 gm. per kilogram by intravenous injection; novasurol was usually curative in a single dose of 0.015 gm. per kilo. In mixtures, however, spirocheticidal activity was increased with smaller amounts of each. For example, 0.007 gm. neosilversalvarsan plus 0.004 gm. novasurol per kilogram usually proved curative, insofar, at least, as to bring about a disappearance of spirochetes in five days with healing of the lesions, which were accepted as the criteria of therapeutic activity in all of his experiments.

I have observed essentially similar results to the extent that the simultaneous use of arsphenamin or neoarsphenamin with a mercurial compound have shown an increase of curative activity in syphilis of the rabbit beyond that exhibited by the arsenical compounds, even though the mercury compound alone was without demonstrable curative activity in the amounts employed.

Combination Therapy of Acute Testicular Syphilis of Rabbits.—In conducting these experiments rabbits were inoculated in the testicles and treated when the lesions were at the acme of activity with numerous motile spirochetes in the secretions. The criteria of comparative spirocheticidal activity were the dissipation of the testicular lesions and the disappearance of spirochetes with inoculation tests of emulsions of lymph-glands removed from the treated animals. The results, however, can be reviewed only in a broad and general manner, as it was not possible to use the very large number of animals required for the frequent repetition of each test in order to secure finer differences in curative activity.

Arsphenamin in dose of 0.005 gm. per kilo caused a regression of lesions but did not produce a complete cure since the minimal curative dose is about 0.012 gm. per kilo; but when given simultaneously with mercurophen in dose of 0.001 gm. or 0.005 gm. of flumerin per kilo, complete cures were generally produced. Arsphenamin mixed with sodium iodid in dose as large as 0.050 gm. per kilo however, did not result in an increase of curative activity.

Similar results were observed with neoarsphenamin. This compound in dose of 0.008 gm. per kilo caused regression of the lesions but not complete sterilization. When administered with mercurophen and flumerin however, complete cures were frequently obtained. In both sets of experiments treatment with mercurophen alone was without any effect upon the lesions while flumerin alone sometimes caused a reduction in the spirochetes with a slight regression of the lesions but never a complete cure.

The results indicate therefore, that mixtures of arsphenamin or neoarsphenamin with mercurophen or flumerin were slightly more effective than the arsenobenzols alone; the same was true to some extent of mixtures of arsphenamin or neoarsphenamin with a water solution of sodium and potassium tartrobismuthate. The addition of sodium iodid in dose corresponding to 3.0 grams per 60 kilograms of weight to the solutions of arsphenamin and neoarsphenamin had no effect however, upon increasing spirocheticidal activity.

Combination Chemotherapy of Chronic Syphilis of Rabbits.—In these experiments rabbits were inoculated in one testicle and castrated ten days later to aid in the dissemination of the spirochetes. Treatment was begun about six to eight weeks later with animals showing cutaneous, muco-cutaneous, lymph-gland or periosteal lesions, etc. The criteria of cure were dissipation of the external and obvious lesions and negative lymph-gland transplants to the testicles of fresh animals.

Arsphenamin (0.005 gm. per kilo) or neoarsphenamin (0.008 gm. per kilo) were injected intravenously once a week for three weeks; at the same time salicylate of mercury (0.001 gm. per kilo) was injected intramuscularly and sodium iodid (0.02 gm. per kilo) administered orally in order to duplicate a commonly employed method for the treatment of chronic syphilis of human beings. In other words the various medicaments were administered separately but at the same time. Whether or not this constitutes combination therapy in the same sense as administering two compounds mixed together in the same syringe, I am unable to state, but at any rate the results have proven of much interest, since combination therapy of this kind is frequently employed in the treatment of chronic syphilis of human beings.

The arsphenamin (total dosage 0.015 gm. per kilogram) and neoarsphenamin (total dosage 0.024 gm. per kilogram) alone served to dissipate the external lesions in the majority of cases, but lymph-gland transplants were usually positive indicating that complete cure had not been accomplished. The salicylate of mercury had some curative effects in that skin and periosteal lesions were improved, but the lymph-gland transplants always yielded positive results. The sodium iodid alone had no appreciable effects.

Rabbits treated with the arsphenamin or neoarsphenamin and salicylate of mercury yielded the highest percentage of complete cures; the simultaneous oral administration of sodium iodid did not have an added curative activity in these experiments insofar as could be determined. The lesions disappeared just as rapidly with arsphenamin and the salicylate as when all three were administered; of course, lesions in some animals disappeared more rapidly than in others, but well-marked variations in individual rabbits did not permit the drawing of finer distinctions and deductions.

Influence of Iodids upon the Treatment of Chronic Rabbit Syphilis with Arsphenamin and Mercury.—Additional experiments were conducted to obtain more definite data upon the curative activity of iodids in chronic syphilis of rabbits when administered with arsphenamin intravenously, salicylate of mercury intramuscularly and mercurial ointment by inunction. Sodium iodid was selected because it is less toxic than potassium iodid.

The intravenous injection of arsphenamin in alkaline solution in dose of 0.008 gm. per kilogram (equivalent to about 0.5 gm. per 130 pounds) once a week for five weeks yielded approximately 60 per cent. of cures in the sense of complete disappearance of the external lesions of syphilis and sterilization of the lymph-glands. When injected along with the

daily administration of sodium iodid by mouth in dose of 0.03 gm. per kilogram, equivalent to 1.8 gm. or 30 grains per 130 pounds, the number of cures was almost the same.

The intramuscular injection of 0.001 gm. of the salicylate of mercury per kilogram, equivalent to about 0.06 gm. or 1 grain per 130 pounds, once a week for five weeks did not yield complete cures; the external lesions improved, but in some instances did not entirely disappear and the lymph-glands were not sterilized. When sodium iodid was given daily at the same time by oral administration in dose of 0.03 gm. per kilogram (equivalent to about 1.8 gm. or 30 grains per 130 pounds, complete cures likewise did not occur, but viewing the group of animals as a whole, the lesions disappeared more rapidly and more animals showed a complete disappearance of all external lesions, than occurred with the group treated alone with the salicylate of mercury.

The daily inunction of 0.06 gm. of 30 per cent. blue ointment (*massa hydrargyri*) per kilogram for 30 treatments, equivalent to about 3.6 gm. or one drahm per 130 pounds, cured approximately 20 per cent. of animals. In all instances the external lesions disappeared with sterilization of the lymph-glands in 20 per cent. The effects observed with these daily inunctions over a period of five weeks were more striking than in the group treated with the intramuscular injections of salicylate of mercury once a week for five weeks, described above.

When sodium iodid was given at the same time in dose of 0.03 gm. per kilogram by oral administration, equivalent to 1.8 gm. or 30 grains per 130 pounds, the percentage of cures was approximately the same. But a comparison of the two groups clinically from week to week showed conclusively that the lesions improved and finally disappeared more promptly among the animals receiving the iodid, although the percentage of animals showing complete sterilization of the lymph-glands was the same in both series.

The evidence of these animal experiments was confirmatory, therefore, of clinical experience, showing that the iodids exert a curative activity in chronic syphilis. The effects were about the same with oral and intravenous injections due, probably, to the rapid absorption of the iodids from the alimentary canal.

Whether the treatment of syphilis with arsphenamin or a mercurial compound and an iodid constitutes an example of combination therapy in the chemotherapeutic sense is uncertain; at the present time the larger part of the curative activity of the iodids is commonly ascribed to their effects upon the ferment-antiferment balance in the blood and tissues, the iodin serving to bind the unsaturated fatty acids and thereby releasing and activating tryptic ferments with the digestion and removal of inflammatory exudates and tissues. If this were the sole activity of the iodids as administered in the treatment of syphilis, their simultaneous use with arsphenamin, neoarsphenamin, mercury or bismuth would not constitute a form of combination therapy in the chemotherapeutic sense. However, it is possible that the iodid likewise exerts a feeble degree of spirocheticidal activity or increases the spirocheticidal activity of the arsenical, mercurial or bismuth compounds and, if the latter were the case, the curative activity of the iodids in syphilis would be due to a combination of effects, one of which would constitute an example of combination chemotherapy.

Advantage and Disadvantges of Combination Therapy in the Treatment of Human Syphilis.—The subject of combination therapy in syphilis is therefore, one of importance in the treatment of this disease. There are relatively few physicians today willing to treat cases exclusively with arsphenamin or its substitutes or with mercury or bismuth alone unless absolutely demanded by peculiar circumstances, as hypersensitiveness to one or more of these compounds. The great majority prefer to follow courses of arsphenamin or one of its substitutes with courses of mercury and the iodids or with bismuth and the iodids. This, however, is not combination therapy as I wish to discuss the subject here; rather I refer to the administration of two or more compounds at the same time either by administering them separately or in mixtures. Probably the oldest and best known type of combination therapy in syphilis is the mixture of bichlorid of mercury and potassium iodid in some suitable vehicle like syrup or sarsaparilla.

Not infrequently chronic syphilis is treated by intravenous injections of arsphenamin or neoarsphenamin combined with mercury by inunction and iodids by mouth; this is probably one form of combination therapy. Quite a number of physicians, however, hesitate to adopt such a method in fear of toxic effects and especially injury of the kidneys by mercury or of the liver by arsphenamin, thereby interfering with elimination and increasing the

chances of producing such serious untoward effects as dermatitis, hepatitis, and nephritis. Wechselmann is largely responsible for instilling this fear into the profession, but he has overemphasized the danger in his desire to blame the accidents of arsphenamin therapy upon mercury rather than upon arsphenamin itself. On the other hand, I do not wish to underestimate the danger; it is reasonable to assume that the simultaneous administration of two compounds capable of producing toxic tissue reactions, like arsphenamin or one of its substitutes along with mercury or bismuth, are more likely to produce toxic effects than any one of these compounds alone; but I believe that with due care in regards to dosage and administration that combination therapy is applicable to the majority of cases without increasing toxic effects and with much better therapeutic results.

Insofar as one may judge by the rapidity of disappearance of spirochetes from human chancres and mucous patches and healing of these lesions, I have not been able to satisfy myself that mixtures of neoarsphenamin and mercury are better than the arsenical alone, although there are numerous reports in the literature indicating that others have found this to be the case. In chronic syphilis, however, I am convinced that much is to be gained in the treatment of many cases by the administration of an arsenical along with mercury or bismuth and with an iodid at the same time.

Linsser's Method of Combination Therapy in Syphilis.—In 1919 Linsser[3] advocated treatment with mixtures of neoarsphenamin and bichlorid of mercury by intravenous injection. His method consists in dissolving 0.45 to 0.6 gm. of neoarsphenamin in 5 c.c. of sterile distilled water and adding 1 to 2 c.c. of a 1:100 solution of bichlorid in water corresponding to 0.01 to 0.02 gm. or $\frac{1}{6}$ to $\frac{1}{3}$ grains; if well borne he later adds as much as 6 c.c. of the bichlorid solution which corresponds to 0.06 gm. or 1 grain, a rather large dose by intravenous injection. These injections are given every 3 to 5 days for courses of 4 to 10 injections.

As soon as the two solutions are brought together in a syringe the color is changed to a very dark greenish-black due to the precipitation of the mercury, the chemistry having been recently discussed by Kolle.[4] It is a most unsightly mixture and one naturally hesitates to inject it. Furthermore, it is difficult to see the "flash" of blood in the syringe as an indication that the vein has been entered and a series of injections tends to produce phlebitis; there are therefore, distinct disadvantages to the method.

Since Linsser published his results, quite a large literature has accumulated upon the use of the method in Germany among which may be mentioned the publications by Gutmann,[5] Holzhauser,[6] Lowenstein,[7] Zirn,[8] Johnson,[9] Oelze,[10] Eicke and Rose,[11] Schmidt,[12] Ebel,[13] Brann[14] and others who claim that the results are generally superior in all stages of syphilis to those observed with similar doses of neoarsphenamin alone and that toxic effects are not enhanced. Bruck[15] however, has advocated the use of novasurol, a double salt of sodium hydroxy-mercury-chloro-phenoxyacetate and diethyl malonylurea, containing 33.9 per cent. mercury, as a substitute for mercuric chlorid, because dissociation is less rapid, with the result that the solutions do not become more than slightly opalescent and yellowish-green within a minute, which permits one to see the flash of blood in the syringe, and because phlebitis is not so likely to be produced. Schonfeld,[16] Biederstedt,[17] Issel,[18] Krebs[19] and others have reported favorable results with this combination as likewise Boas and Pontoppidan,[20] Krug,[21] Oelze,[22] Bab[23] and others with "cyarsal," the potassium salt of a nuclear mercurated oxybenzoic acid. Rebandi[24] reports favorably upon the use of "neobiol," an organic combination of bismuth and arsenic in a benzol ring, in dose of 0.1 to 0.3 gm. by

intravenous injection. I have employed mixtures of neoarsphenamin and mercurophen; also neoarsphenamin and flumerin, the technic being described in Chapter LXII.

There is, therefore, quite a large and favorable literature upon combination therapy in syphilis and I believe the subject is worthy of more consideration than it has received in this country. A great disadvantage is the summation of toxic effects, but, as I may affirm from clinical experience, this is more theoretical and fanciful than real. It is true that each case of syphilis must be decided upon individual conditions; for example, I would not consider combination therapy in the presence of kidney and liver disorders in fear of accidents due to faulty elimination. Even in the treatment of acute early syphilis, I believe something better is to be gained by giving one of the arsenicals intravenously while mercury is given by inunction than when the arsenicals alone are given; if inunctions are not convenient or possible I do not hesitate to inject the salicylate or succinimid intramuscularly. If these are objected to, I combine neoarsphenamin with mercurophen or flumerin in the same syringe and inject intravenously, as described in Chapter LXII. In the treatment of chronic syphilis I believe the indications and results are even better and especially in "Wassermann-fast" cases, due apparently to acquired arsenic resistance. In cases selected with due care, I have not seen any ill effects and belive that the results are better with shorter combination courses than with the usual long drawn out courses of arsphenamin or one of its substitutes followed by mercury. But this is too important a subject to be discussed with a few words and will be considered with more detail in chapters devoted to the treatment of chronic syphilis; here I merely wish to record my opinion that combination therapy is worthy of serious consideration for improving therapeutic results and especially in chronic syphilis, and with due care in the selection of cases, drugs, doses, intervals of administration and length of "courses" it is a harmless method from the standpoint of untoward reactions.

Stokes,[25] in writing of the combination of mercury with arsphenamin in the treatment of syphilis, states that "to secure the maximum combination of benefits, with the minimum of defects from both drugs, I believe it is essential, especially in early cases, to use them simultaneously rather than alternately. Prolonged observation of the effect of this combination has made me believe them actually synergistic in effect, and without permanent damaging effect on the excretory mechanism." Stokes, however, is against the use of intramuscular injections of insoluble salts of mercury, because the formation of mercurial depots of unknown capacity in the tissues of patients of unknown resistance and tolerance in an adventure in speculative therapy that his experience with the kidneys in syphilis will not countenance.

Limitations of Arsphenamin and Its Substitutes Alone in the Treatment of Syphilis.—Certainly the treatment of syphilis with arsphenamin or its substitutes alone has proven unsatisfactory. The hopes originally entertained of the curative action of arsphenamin alone in syphilis have not been realized and especially in chronic syphilis; undoubtedly, many cases treated in the early stages with arsphenamin and neoarsphenamin alone are being cured and the drugs have likewise proven efficacious in the prophylaxis of syphilis, but in chronic syphilis the tendency is always to include mercury in treatment. Furthermore the intensive use of arsphenamin alone is more dangerous from the standpoint of toxic reactions than milder dosage made possible by combination therapy; it is also more likely to produce neural or other Jarisch-Herxheimer reactions than mercury and the latter should always

preceed as the advance guard of therapeutic attack in the treatment of neurosyphilis and chronic syphilis in general.

Limitations of Mercury, Bismuth and the Iodids Alone in the Treatment of Syphilis.—But if it is unwise to depend upon arsphenamin and its substitutes alone for the treatment of any but the earliest cases of syphilis, the same may be said of mercury. In acute early syphilis it is apparently not possible to give enough mercury to produce such prompt spirocheticidal effects as to prevent considerable dissemination of the spirochetes; the lesions are dissipated but not as rapidly as by adequate doses of arsphenamin. To-day we should not be satisfied with the results secured by Fournier in the days of treatment with mercury and iodids alone since it is entirely likely that the incidence of tertiary syphilis was higher than he thought in view of the advances made during the past twenty years in the serological and clinical diagnosis of chronic syphilis and especially of the central nervous system. Undoubtedly good and even splendid results were secured by Fournier and other syphilologists of experience with mercury in the pre-arsphenamin days, but not as good as they believed although mercury is unquestionably curative in syphilis, in the strict sense of the term and not merely a simple "obliterator of lesions" or a "whitewasher" of symptoms.

To treat syphilis with the iodids alone is out of the question, except in those occasional cases of tertiary syphilis of aged individuals to whom relief from a gumma is all that can be hoped for without involving the possibility of effecting a cure; likewise in the treatment of cases of tertiary syphilis to whom mercury, bismuth and the organic arsenicals cannot be given because of a general break down of the cardiovascular and eliminative organs, but who are urgently in need of relief from a threatening gummatous lesion. Neither are we justified in relying upon mercury alone or bismuth alone if it is possible to give one of the organic arsenicals in addition. In short, best results for the majority of cases are secured by a judicious combination of these remedies. It is not a question of arsphenamin *versus* mercury or the reverse, according to one's prejudices; both classes of compounds are capable of doing both harm and good. What is needed is a good working knowledge of both including bismuth and the iodids, so that the patient rather than the disease may be treated in the manner best suited for each individual case.

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CHAPTER XXXI

CHEMOTHERAPY OF FRAMBESIA TROPICA, RELAPSING FEVER, RAT-BITE FEVER, VINCENT'S ANGINA, GINGIVITIS, PULMONARY SPIROCHETOSIS, AND OTHER SPIROCHETIC AND LEPTOSPIRA DISEASES OF MAN AND THE LOWER ANIMALS

As reviewed in preceding chapters, arsphenamin and neoarsphenamin have been found very efficacious remedies for various spirochetic infections besides syphilis and notably in frambesia tropica and relapsing fever. These compounds and the organic arsenicals, as a group, are general spirocheticides and have been found valuable in the treatment the various spirochetic infections of man and the lower animals. Indeed, as in frambesia, the results have been sometimes more brilliant and satisfactory than those obtained in syphilis, and the purpose of the present chapter is to discuss the specific therapy of these various non-syphilitic spirochetic infections of human beings.

Chemotherapy of Frambesia Tropica.—Shortly after the discovery of salvarsan (arsphenamin), Nichols[1] found that it had a remarkable curative effect in the treatment of rabbits experimentally infected with the disease (*Sp. pertenuis*), and almost simultaneously Strong[2] and Castellani[3] observed extremely satisfactory results in the treatment of human cases, the former observing in the treatment of twenty-five cases with single injections, that the granulomatous lesions began to recede in three to four days and generally disappeared entirely in ten to twenty days. These results were soon confirmed and, indeed, the curative effects of arsphenamin and neoarsphenamin have been proven so remarkable that many physicians have expressed the opinion that Ehrlich's dream and hope of a *therapia sterilisans magna*, the complete cure of a disease by a single injection of drug, has been realized in many cases of this infection.

As recently stated by Fox,[4] frambesia tropica does not occur in the United States except in imported and isolated instances at seaports, despite the fact of its prevalence in the West Indies. Goodman,[5] Schamberg and Klauder[6] and others have reported isolated cases in which the lesions were rapidly dissipated and the positive Wassermann reactions reduced to negativity by intravenous injections of neoarsphenamin. The disease, however, is prevalent in the West Indies, Central America, northern part of South America, Africa, Philippines, Guam, Porto Rico, etc., and the wonder is that it is not more frequently seen in the United States, although it is said never to occur in the mountains and cold districts and rarely above an elevation of 800 feet.

The disease is known by various names in different localities, such as "yaws" in the English colonies; "pian," in the French colonies; "bolobo" in the Belgian Congo and "bouba" or "boubas," in the Spanish domains. By some physicians it is believed to be a tropical form of syphilis, but it is generally regarded at present as a separate disease due to a closely related spirochete. The infection is not transmitted by sexual congress in more than 1 per cent. of cases and is not transmissible to the offspring. The primary lesion, "mother yaw" or "madre buba," generally occurs on the extremities and infection prob-

ably requires a break in the epithelium by trauma or the bites of insects and especially by flies. The generalized eruption follows in a few months and is characterized by widespread granulomatous lesions which may undergo ulceration with a great deal of deformity. Smears of these lesions generally show an enormous number of spirochetes, which is in sharp contrast to the few spirochetes in secondary syphilitic skin lesions.

In the majority of cases, the lesions of frambesia appear to be confined to the skin, mucous membranes and bones, and this probably accounts for the fact that it is not transmissible from parents to children, since the generative organs do not become involved nor do periods of spirochetemia develop, although Harper[7] claims that it has produced abortions and miscarriages among the women of the Fiji islands, where almost every one contracts the disease and where treatment with arsphenamin and neoarsphenamin has yielded brilliant results. The internal viscera, however, do not always escape and most physicians experienced in the disease believe that a tertiary stage may develop with lesions in the internal organs. Castellani regards "*gangosa*," or rhinopharyngitis mutilans, an ulcerative condition of the palate, nose, pharynx, and skin surfaces of the body, a probable late sequela of frambesia, which slowly spreads to the nose and larynx, destroying cartilage and bone, and causing much deformity. Kerr[8] believes it is either a tertiary frambesial or syphilitic lesion. Even the central nervous system may be apparently involved in exceptional cases with spinal fluid changes and symptoms resembling locomotor ataxia and other syndromes resembling syphilitic infection of these tissues, as reported by Chatellier,[9] Hunt and Johnson,[10] and others. It is possible, however, that in some of these cases syphilis was present. Indeed, Parham[11] and not a few of the Army surgeons, believe that frambesia is a form of syphilis, although, as stated above, the weight of opinion is against this view. At any rate, the lesions are largely cutaneous and this accounts for the rapidity of cure just as the skin lesions of syphilis are usually rapidly cured by treatment with arsphenamin and neoarsphenamin. Ulcerated lesions, however, may require more prolonged treatment as originally advised by Strong, since the spirochetes may be well enmeshed in inflammatory fibrous tissue and exudates. *Sp. pertenuis*, however, does not produce the same degree of perivascular exudations and thickening of blood-vessel walls as does *Sp. pallida*, and this circumstance greatly aids in the cure of the disease by arsphenamin and neoarsphenamin.

From the standpoint of specific therapy it may be stated that mercury is almost without effect. Potassium iodid is helpful in deep seated and chronic lesions, especially those involving the bones, but it is slow in action and but feebly spirocheticidal, although in combination with tartar emetic it may yield good results. Castellani[12] has used a combination of this kind according to the following formula, as a substitute for arsphenamin treatment:

Tartar emetic	gr. i
Potass. iodid	℥i
Sod. salicyl	gr. x
Sod. bicarbon	gr. xv
Glycerin	f℥ ii
Water	q.s. f℥i

This dose (one ounce diluted with three ounces of water) is given to adults three times a day, children receiving one half as much, for ten to fifteen days; it is then discontinued for one week and repeated again for ten to fifteen days. Guerro and his colleagues,[13] as well as Viswalingham[14] and others, have reported good results with this therapy.

But by far the best results have been observed with arsphenamin and neoarsphenamin, as shown by a host of reports, among which may be mentioned, in addition to those already cited, those by Flu,[15] Alston,[16] Koch,[17] Scherschmidt,[18] Cockin,[19] Brochard,[20] Baetz,[21] Reed,[22] Girling,[23] McDonald,[24] Bergen,[25] de Boissiere[26] and Tournier.[27] These reports, from different parts of the world and embracing the treatment of thousands of cases, are without exception most favorable to the treatment of the disease with arsphenamin and its substitutes. In the earlier work, intramuscular injections were given, but these were soon replaced by intravenous injections and the reports on treatment with different brands of neoarsphenamin appear to be as good and satisfactory as with different brands of arsphenamin. One of the most

recent reports is by Moss and Bigelow,[28] of a commission sent by the School of Tropical Medicine of Harvard University to the Dominican Republic. Of 362 cases treated with a *single* intravenous injection of 0.6 gm. neoarsphenamin:

20 per cent. were cured or practically cured.
59 per cent. were improved.
21 per cent. were not improved.

But cases receiving three or four injections were cured or improved in the majority of cases, as shown in the following results with 39 cases:

44 per cent. cured or practically cured.
54 per cent. improved.
2 per cent. not improved.

Recently Paterson[28a] has reported favorably upon the results of treatment with bismuth, the salt employed being sodium and potassium bismuth-tartrate in dose of 3 grains for the adult injected intramuscularly in 2 c.c. of sterile water. Probably the oily suspensions would prove better from the standpoint of producing less local reaction and pain and doubtless a series of injections at intervals of 5 to 7 days are required; I suggest the following plan of treatment for this disease:

1. The same general precautions against toxic reactions are required as in the treatment of syphilis. When conditions permit, the urine should be examined and the Wassermann reaction conducted.

2. Four to six weekly injections of arsphenamin in dose of 0.4 to 0.6 gm., or neoarsphenamin in dose of 0.6 to 0.9 gm., for adults by intravenous injection; children to receive smaller amounts proportionate to weight. If the lesions are ulcerative and involving bone, potassium iodid may be given at the same time by ingestion.

Sulpharsphenamin has not yet been reported upon in this disease, but in consideration of the excellent results observed in syphilis, and since it may be given by subcutaneous and intramuscular injection which are always easier than intravenous injections in the tropics amidst the unhygienic and difficult conditions surrounding centers for the treatment of the disease, I believe it is worthy of trial in dose of 0.6 gm. (0.3 gm. in each buttock) once a week for at least four to six weeks.

If conditions are such that multiple injections cannot be given, I would not hesitate to inject adults in good general condition with 0.8 gm. of arsphenamin or 1 gm. of neoarsphenamin by intravenous injection, or 0.6 g. of sulpharsphenamin by intramuscular injection (0.3 gm. in each buttock), and follow this up if possible by the internal administration of Castellani's mixture.

3. After the course of injections, a rest of one month may follow when the Wassermann test should be repeated. If a positive reaction is observed, an additional series of injections should be given.

It is not necessary to examine the spinal fluid, since infection of the central nervous system occurs so rarely, unless there are clinical signs of involvement.

The criterion of cure has been the disappearance of the outward manifestations of the disease and since visceral involvement is exceptional and transmission to offspring known not to occur, this would appear sufficient. But when conditions permit, the Wassermann test by a sensitive method should be included and specific treatment continued until a state of negativity is obtained over a period of at least one year.

Chemotherapy of Relapsing or Recurrent Fever.—Since a great deal of the fundamental chemotherapeutic investigations by Ehrlich and Hata were done

with mice infected with *Sp. obermeieri*, it is no wonder that human beings suffering with relapsing or recurrent fever were among the very first to benefit from the discovery of salvarsan (arsphenamin). Apparently all species of the spirochete of relapsing fever are highly susceptible to arsphenamin and neoarsphenamin, since complete cures have been recorded from single injections in the treatment of infections with *Sp. obermeieri* (European relapsing fever), *Sp. duttoni* and *Sp. kochi* (African relapsing fever), *Sp. carteri* (relapsing fever of India), *Sp. barbera* (relapsing fever of northern Africa) and *Sp. novyi* (American relapsing fever). The disease, therefore, is widely distributed and isolated cases have been reported in the United States by Briggs[29] and others.

Following encouraging laboratory experiments with atoxyl and other pentavalent arsenicals, Glaubermann[30] employed atoxyl in the treatment of human infections and Iversen[31] and others used arsacetin; but owing to the great superiority of arsphenamin and neoarsphenamin from the standpoint of lesser toxicity and greater spirocheticidal effects, these drugs are no longer employed, although Mante[32] has used arrhenal and Portocalis[33] has used galyl with some success.

Iversen[34] was first to report upon the arsphenamin treatment of human cases of the disease and in a series of 55 observed an apparent cure in 92 per cent. following the intramuscular injection of a single dose of 0.2 to 0.3 gm. of salvarsan (arsphenamin). Relapses occurred in 4 or 8 per cent. of the cases and one cured case was later re-infected. Iversen noted a disappearance of the spirochetes from the blood in four to ten hours after injection and in the majority a complete sterilization resulted, fulfilling Ehrlich's prediction of successful *therapie magna sterilisans*. At the same time, Bitter and Dreyer reported the successful treatment of thirteen cases with arsphenamin and since then Smirnoff,[35] Iversen,[36] Jarno,[37] Robledo,[38] Manson and Thornton,[39] Saceghem,[40] Prussian,[41] Lorentz,[42] Zeller[43] and others have reported excellent results with arsphenamin and neoarsphenamin, although Peacock[44] claims that arsphenamin frequently fails to cure the disease. As stated in Chapter LXXVI individuals with paresis and other types of neurosyphilis have been treated by artificial inoculation with relapsing fever as a substitute for malarial inoculation and these infections are said to be promptly cured by the administration of arsphenamin or neoarsphenamin.

For treatment either arsphenamin or neoarsphenamin may be employed by intravenous injection. Sulpharsphenamin has not yet been reported upon, although I have found it curative by subcutaneous, intramuscular and intravenous injection in mice experimentally infected with *Sp. novyi*. Very probably it will find a useful place in the treatment of relapsing fever in Africa and other localities where subcutaneous or intramuscular injections can be more readily given on a larger scale than intravenous injections. The following method of treatment may be employed:

1. The intravenous injection of 0.6 gm. arsphenamin or 0.9 gm. neoarsphenamin during the attack when spirochetes are present in the blood. Best results have been observed when treatment was given while the fever was mounting rather than during the periods of intermission. If the patient is first seen during the afebrile period, it is advised to wait until a paroxysm is developed before administering the drug.

2. The patient should be kept under observation, with frequent examinations of the blood, and given another injection if parasites are found or a paroxysm occurs.

3. According to most observers only one or two doses are required, but in long standing infections a series of injections may be necessary. Since the spirochetes may be rendered "arsenic-fast," it is well to treat with large doses as required, rather than small doses at frequent and close intervals.

Chemotherapy of Rat-bite Fever.—That the bite of an animal, especially the rat, may cause infection in man with different microörganisms is obvious to anyone who makes a careful survey of the literature of diseases produced by bites of the rat, mouse, cat, dog, ferret, weasel, etc. In Japan a paroxys-

mal febrile infection of the relapsing type frequently follows the bite of rats, and similar cases have been observed in the United States and Europe. The wound heals readily, but after a variable incubation period of a few days to a month it becomes inflamed and painful. Lymphangitis and adenitis develop and are quickly followed by symptoms of systemic infection ushered in by a chill and rapid rise in temperature. There are extreme prostration, severe generalized muscular pain, headache, weakness, and loss of appetite. Stupor, delirium, and even coma may supervene. There is muscular tenderness and rigidity and the tendon reflexes are frequently exaggerated. A characteristic exanthem of bluish red, erythematous, sharply marginated macules appear of various sizes and of general distribution. After five to nine days the temperature falls by crisis accompanied by a profuse sweat and all symptoms subside. The disease then assumes the relapsing type with paroxysms occurring at fairly regular intervals, usually about once a week. The course may vary from one to three months or even longer. Gradually the relapses become less frequent and less severe and the disease often terminates with an abortive paroxysm. The more important complications are nephritis, severe anemia, and emaciation. About 10 per cent. of the cases terminate fatally, usually during the first febrile period, occasionally later from nephritis or exhaustion. The Wassermann reactions have been reported as uniformly negative.

That rat-bite fever is an infectious disease dependent upon the introduction of a specific organism or virus at the site of the bite seems highly probable. There has been no definite evidence presented to show that the rat inflicting the bite was in any way diseased or other than a normal rat. In view of this negative evidence it does not seem improbable that the etiological agent is simply a saprophyte in the mouth of the rat. Individual susceptibility seems to play a definite part in the contraction of the disease, as instances are recorded in which the second of two people bitten by the same rat has been the only one to develop rat-bite fever. Nixon[45] has reported a case similar to rat-bite fever following the bite of a ferret, and Schottmuller[46] a case incident on the bite of a South African squirrel. That the etiological factor in such cases is closely related to that occurring in rat-bite fever is possible. I may state, however, that the bite of the white rat in this country is apparently without danger of producing the disease; at least, my assistants and I have been bitten innumerable times during the past ten years but in no instance have there followed the febrile attacks of rat-bite fever.

Various microörganisms have been regarded as causing the disease, the etiology being still uncertain, and it is highly probable that at least two forms of the disease may exist, namely, a spirochetic type due to *Spirochæta morsus muris*, discovered by Futaki, Takaki, Taniguchie and Osumi[47] and especially prevalent in Japan, and a type due to *Streptothrix muris ratti*, discovered by Schottmüller, and found in Europe and the United States by Blake,[48] Tunnicliff[49] and Tileston;[50] Arkin,[51] in a recent review of the literature on the disease, believes that the spirochete is to be regarded as the primary etiologic agent.

Arsphenamin, first employed by Hata[52] in 1912 in the treatment of eight cases of the disease, is commonly regarded as the best available remedy, but not necessarily as a specific. Bergamini,[53] Tileston,[54] Delal,[55] Surveyor,[56] Powell,[57] Solly[58] and Burton-Fanning[59] have reported successful treatment with arsphenamin and neoarsphenamin, and two additional cases have been recently reported by O'Leary,[60] who has especially described the skin lesions of the disease, successfully treated with arsphenamin. Borelli[61] states that mercury has curative activity, but this statement has not been confirmed, arsphenamin and neoarsphenamin being the remedies of choice.

If the wound is seen immediately it should be cauterized and this procedure is stated to prevent the disease if applied within an hour. It may be possible that the thorough application of 33⅓-per cent. calomel ointment will likewise act as a prophylactic, although I do not know of any instance in which this has been tried. From 3 to 6 injections of arsphenamin or neoarsphenamin are usually given at five to seven day intervals, followed by one or

two more after the subsidence of all symptoms, to prevent a relapse. Following the first injection, a Herxheimer-like flare-up may occur, with elevation of temperature and transitory accentuation of the lesions.

Chemotherapy of Vincent's Angina.—The cause of Vincent's angina is now quite universally accepted as being *B. fusiformis* and a spirochete designated as *Sp. vincenti.* Whether the bacillus is a form of the spirochete, or a separate organism, cannot be stated; nor is it certain that the spirochete is a separate entity, since it may be one of the several species to be found about the teeth and gums. Spirochetes and fusiform-like bacilli are quite commonly found in the tartar deposits and decayed food debris of the teeth, and, while ordinarily saprophytic, are apparently capable of exciting inflammatory changes and tissue necrosis, especially when the resistance of the tissues is lowered by gingivitis and stomatitis, due to gastro-intestinal disturbances from scorbutus and other dietary and metabolic errors, to the elimination of mercury or to plain neglect of oral hygiene with tremendous proliferation of streptococci, various bacteria, yeasts and fungi known as oral sepsis. In other words, we may have low grade inflammatory changes in the gums apparently caused by spirochetes, fusiform bacilli and other organisms; in exceptional instances, and especially in children, the fusiform bacilli and spirochetes are capable of exciting an acute ulcero-membranous infection of the tonsils and uvula, sometimes involving the gums and sides of the tongue, and accompanied by adenitis, designated as *Plaut-Vincent's angina.* The disease is commonly mistaken for diphtheria and is infectious, epidemics sometimes occurring in institutions and among soldiers. Thus Bouty,[62] Campbell and Dyas,[63] Tixier and Tobe[64] and others have described small epidemics in the recent World War in which the incidence among soldiers has been as high as 23 per cent. The infection may also extend to the nasal cavities and even to the accessory sinuses: I have seen a fatal case in the child of four years due to infection of the antrum of Highmore on the left side with extension into the orbit. These nasal infections, however, should not be mistaken for Castellani's spirochetic rhino-pharyngitis due to *Spiroschaudinnia minuta*, which is a rare disease and to the best of my knowledge not reported so far in America.

The bacteriological diagnosis of Vincent's angina is not ordinarily a difficult matter and is made by the examination of smears and not by cultures. Smears may be stained with hot diluted carbol fuchsin (1:5) for a few seconds, which serves to bring out the spirochetes and curved, banana-shaped, granular, fusiform bacilli.

Various local applications have been advocated from time to time for the treatment of this infection, among which may be mentioned applications of solutions of copper sulphate, potassium chlorate, iodin, chromic acid (2-per cent.), etc.; also powdered methylene-blue, Dakin's solution, acriflavine, etc. Arsphenamin and neoarsphenamin, however, have yielded particularly good results and especially by local application. As stated in Chapter XXVIII, both arsphenamin and neoarsphenamin are undoubtedly spirocheticidal by local contact with *Sp. pallida* and I have found both drugs capable of destroying the mouth spirochetes as well, when brought in contact with them in the secretions. Whether or not these compounds are destructive for the fusiform bacillus cannot be stated.

Intravenous injections of arsphenamin and neoarsphenamin do not ordinarily influence the spirochetes of the mouth. I have found, for example, that fuso-spirillar gingivitis in syphilitic individuals under treatment with these compounds, is apparently not influenced, but in severe and extensive *Vincent's angina,* intravenous injections are apparently efficacious because

of the deep seated lesions, as indicated by the reports of Tixier and Tobe, Greene,[65] Spano,[66] Morgan,[67] Hirsch,[68] Bruggeman[69] and others; I have also found them efficacious in the treatment of Vincent's infections of the bronchi. Barenberg and Bloomberg[70] have recently described the successful treatment of Vincent's angina of children with one or two intramuscular injections of sulpharsphenamin combined with local applications of the drug, and practically all of the previous mentioned writers, in addition to Reckford and Baker,[71] Citron,[72] Amsden,[73] Gougerot, Goullier and Vaslin,[74] Zilz,[75] Voss,[76] Achard,[77] Renon and Desbouis,[78] Niglot and Levy[79] Rolleston[80] and others, have reported successful results from treatment with local applications of arsphenamin or neoarsphenamin. For the treatment of fuso-spirochetal ulcero-membranous angina these compounds may be regarded as specifics, insofar, at least, as concerns the part played by spirochetes. Recently Driscoll[81] and Vonderlehr[82] have reported excellent results in the treatment of Vincent's angina with intravenous injection of 1 per cent. solutions of tartar emetic in dose of 5 to 10 c.c. repeated if necessary every two to three days, severe cases requiring about six injections. Driscoll states that he has also employed this drug with success in the treatment of "trench mouth."

The specific treatment of Vincent's angina may be summarized somewhat as follows:

1. In severe cases neoarsphenamin may be injected intravenously or sulpharsphenamin intramuscularly, the doses for adults being 0.9 and 0.4 gm. respectively, children receiving less in proportion to body weight. Two to four injections at intervals of 4 or 5 days may be required to check the disease, subsequent treatment being by local applications. If these fail it may be well to try tartar emetic by intravenous injection in dose of 5 to 10 c.c. of a sterile 1 per cent. solution.

2. Local applications are always required in addition, and for the usual and milder infections may alone suffice for treatment. The method of local treatment has been described in Chapter VIII, but a choice may be made of any of the following preparations: (*a*) 2-per cent. solutions of arsphenamin or 4-per cent. solutions of neoarsphenamin in 50-per cent. glycerin; (*b*) 10-per cent. suspensions of arsphenamin or neoarsphenamin in pure glycerin; (*c*) the powder of either drug applied by swabs dipped in glycerin.

It is necessary to apply the chosen preparation very thoroughly, and after preliminary cleansing by means of swabbings and gargling with diluted *liquor antisepticus* or any other suitable antiseptic solution. The preparations may gradually turn darker in color, owing to oxidation, but this does no harm until a very dark green color results in which case oxidation has probably proceeded to the stage when the preparation loses in spirocheticidal activity and should be renewed. My favorite preparations are the 2- to 4-per cent. solutions in 50-per cent. glycerin, but an equally good method is to take a 0.6 gm. tube of neoarsphenamin, file off the tip, add 6 c.c. of glycerin, mix well to secure an even suspension and apply two or three times a day with cotton applicators until evidences of healing are apparent, when one or two applications per day may suffice until healing is complete. In addition to these applications it is sometimes advisable to apply a 2-per cent. solution of chromic acid, once or twice a day by means of an atomizer.

Chemotherapy of Gingivitis or Pyorrhea Alveolaris.—The importance of gingival infections is universally recognized by both dental and medical professions, not only in relation to the purely local effects in the mouth, but also in relation to focal infection or the development of general pathological conditions as anemia, acute and chronic arthritis, neuritis, vascular, myocardial and renal degenerations and inflammations.

In the majority of cases the disease apparently begins on the surface of the gums (*a gingivitis*), infection being aided by mechanical injury of the tissues and neglect of oral hygiene. When neglected, the inflammation advances along the root of the tooth into the alveolus, destroying the tissues of the peridental ligaments and producing thereby a series of suppurative cavities or pyorrheal pockets, exposing the cementum and finally causing its absorption (*chronic suppurative pericementitis*) with suppuration, necrosis and absorption of the alveolar processes (*chronic suppurative alveolitis*), resulting in the loosening and loss of teeth.

These changes may be and usually are, associated with abscesses at the roots of one or more teeth (apical abscesses), although these infections are usually caused by direct exposure of the root canals by cavities or, in the absence of these, possibly, in some cases, by infection of the periapical tissues by organisms brought to the parts by the blood or lymphatic channels.

The mouth constantly harbors various bacteria, fungi and some protozoa, notably *Endameba buccalis* and different varieties of spirochetes. Among the bacteria, staphylococci, streptococci, pneumococci and *B. acidophilus* are usually to be found and all of these are capable of inducing low grade inflammatory changes when their invasion into the tissues of the gums is aided by abrasions due to picking and brushing of the teeth, by accumulations of tartar or by minute injuries from other causes; colonies of bacteria and molds growing between the teeth in tartar deposits may produce sufficient amounts of toxins to cause superficial erosions and thereby aid infection of the deeper tissues. As shown by Smith and Barrett, *Endameba buccalis* may be present and by means of its migratory and digging movements, carry bacterial and spirochetic infection into the deeper tissues. No doubt diet deficiency and various systemic and metabolic disturbances are to be regarded as etiological agents of importance in the production of gingivitis, but sooner or later bacterial or spiro-fusillar infections become factors of secondary importance and in some cases at least, may be indeed the primary cause. The chemotherapy of gingivitis is therefore, directed against this primary or secondary infection.

In all cases of chronic suppurative gingivitis bacterial infection may be said to play an important part. In many cases, however, examination shows such a preponderance of spirochetes, with or without fusiform bacilli, that these organisms are to be accepted as playing the major role in infection and especially in "trench mouth." Endamebas may be found in both bacterial and spirochetic types of the disease, but they are not to be considered a cause of the infection but important secondary invaders capable of carrying infection into the deeper tissues. Various molds and yeasts are usually found, but their importance has never been estimated and it is highly probable that they are of secondary importance.

The proper treatment of gingivitis or pyorrhea alveolaris, therefore, requires a bacteriological diagnosis for the purpose of determining whether the infection is purely *bacterial* (the usual normal or but slightly increased flora of spirochetes and molds being also present), or predominently *spirochetic* (some bacteria being always present). At the same time a search is made for endamebas, for if present in increased numbers, adequate measures for their destruction may be required. In the majority of cases these examinations disclose an increase of both bacteria and spirochetes and local treatment should be directed against both of these in addition to the usual surgical procedures. This differential bacteriological diagnosis may be made by the examination of simple smears of gingival material.

From the standpoint of chemotherapy it is necessary to approach the subject of treatment from that of specific chemical disinfection of the tissues by both the local and systemic administration of parasiticidal agents. Unquestionably the subject of local disinfection is the more important of the two; indeed, from the standpoint of systemic medication, only the possibility of influencing spiro-fusillar infections with intravenous injections of arsphenamin and its congeners, or of tartar emetic, as recommended by Driscoll[83] and mentioned above in the treatment of Vincent's angina, are worthy of consideration. Naturally a host of chemical agents have been proposed for the local disinfection of the gums and I have recently described the results of my bactericidal and spirocheticidal tests with a number of these in relation to the chemotherapy of gingivitis.[84]

I well realize that local disinfection of the gums is only a part of the required treatment but I believe that its importance and possibilities as an adjuvant to surgery, the administration of vaccines, etc., is worthy of closer attention. This would appear to be particularly true in the treatment of incipient cases as likewise in the nature of a "follow up" treatment to prevent relapses of the disease. I well realize that in severe cases with pyorrheal pockets involving the destruction of considerable peridental membrane and periosteum with absorption of considerable alveolar process, that surgery is especially indicated, but even in these the application of suitable disinfectants would appear to be useful as a form of adjuvant therapy.

But unquestionably the most important single factor in the chemotherapy of gingivitis, is frequency of application of the chosen disinfectant. When one remembers that within a few hours bacteria and spirochetes are capable of proliferating greatly and contaminating the treated tissues, it is apparent that frequent applications are indicated in the treatment of gingivitis, as is true of the local treatment of infections of the eyes, of wounds and other tissues and organs of the body. Indeed, I believe that failure in the treatment of gingivitis is due much more to this factor than to a lack of suitable disinfectants.

For the majority of cases of gingivitis local applications of a disinfectant will probably continue to be the usual practice insofar as chemotherapy is concerned. It is true that a large literature indicates that the intravenous injection of arsphenamin or neoarsphenamin or the intramuscular injection of sulpharsphenamin may prove helpful in the treatment of spiro-fusillar infections (Vincent's angina and "trench mouth") but these cases are only a small part of the total. I have observed many cases of spirochetic gingivitis among individuals who had received as many as twelve or more intravenous injections of arsphenamin or neoarsphenamin and it is my opinion that the intravenous administration of these anti-syphilitic arsenicals does not benefit gingivitis unless the gingival lesions are syphilitic or possibly a severe Vincent's infection.

Furthermore, the intravenous injection of tartar emetic in the treatment of spiro-fusillar gingivitis is still under trial and the subcutaneous injection of emetin hydrochlorid has not only failed to fulfill early expectations but is usually contraindicated by the slight importance to be attached to the presence of *Endameba buccalis* as likewise by reason of the possibility of disagreeable and even dangerous toxic reactions when adequate amounts are administered. Since stovarsol is apparently proving efficacious in the treatment of amebic dysentery it may be worthy of trial in the treatment of gingivitis, when so many amebae are found in the pyorrheal pus as to demand something for their destruction. Possibly the local application of solutions of this compound to the gums may be worthy of trial combined with the internal administration of 0.5 gm. in capsules two or three times a day for three to six days.

Certainly chemotherapy has nothing to offer in the way of systemic medication for the treatment of bacterial infections of the gums or for sterilizing the pus at the apex of a tooth. Even though the majority of organic and inorganic salts of mercury possess a high bactericidal and spirocheticidal activity *in vitro* and may be deposited or eliminated in the gums as a sulphid following internal administration, it is well known that this compound not only fails to influence the bacterial and spirochaetic flora of the gums but may actually aggravate the course of a gingival infection.

Local applications therefore, continue to be, for the present at least, of most importance in the disinfection of the gums. A host of substances have

been used but with the exception of the mercurial and silver salts and iodin, these are usually very low in parasiticidal activity although agreeable to the taste and otherwise elegant pharmaceutical preparations. Barrett and others have used local applications of 1 per cent. arsphenamin or 2 per cent. neoarsphenamin in the treatment of spirochetic gingivitis with some success, as would be expected because of the spirocheticidal activity of these compounds, but arsphenamin and neoarsphenamin are too low in bactericidal activity to be used alone in the majority of cases and I believe that mercurophen and metaphen are of more promise because of their higher spirocheticidal and bactericidal activity as well, presumably, of a high amebicidal activity. It is easily possible however, to use a mixture of arsphenamin or neoarsphenamin by taking into a syringe equal parts of a 1 to 2 per cent. solution of the former and a 1:500 stock solution of either mercurophen or metaphen. Mercuric chlorid and cyanid however, cannot be used in this way because of a precipitation of mercury.

But the method of application is of more importance. Preliminary cleansing and removal of tartar deposits with a general surgical overhauling are of prime importance. Pockets must be treated by the dentist and for this purpose I would suggest the instillation of 1:500 solutions of either mercurophen or metaphen alone or in combination with arsphenamin solution as compounds fulfilling acceptably the essential properties of a local disinfectant. The frequency of application will depend upon the individual case but in the majority will probably demand the services of the dentist daily for a time at least.

But it is urgently required that dentists work out a method for keeping the disinfectant in longer contact with the tissues. Merely to apply a water solution of arsphenamin, mercurophen or metaphen is almost sure to be followed in a few minutes by high dilution by the saliva, a very brief contact and re-contamination of the tissues probably in a matter of ten minutes.

In the disinfection of pyorrheal pockets it would appear advisable to incorporate 1:500 mercurophen or metaphen into some sort of a jelly or paste capable of being longer retained than a solution. Such are readily prepared and suitably flavored to mask the metallic taste of the disinfectant. In the treatment of spiro-fusillar and spirochetic gingivitis, pastes of arsphenamin prepared with water or glycerin or the powder itself have been applied but I believe solutions are to be preferred and especially one employing 1 per cent. arsphenamin in 50 per cent. glycerin, prepared as follows:

Arsphenamin	0.3 gm.
Hot water or 50 per cent. glucose solution	15.0 c.c.
Dissolve and add Glycerin	15.0 c.c.

This solution gradually becomes dark in color due to the production of oxidation products, but it maintains a high degree of spirocheticidal activity until a deep blackish-green color occurs.

But since it is known that spirochetes may acquire a resistance or fastness to the arsenicals *in vitro*, it would appear inadvisable to use arsphenamin alone over a long period of time; possibly better results may be secured by using it alternately with one of the mercurials mentioned.

But a daily local treatment by the dentist is insufficient. The patient should be carefully instructed in a co-operative treatment to be applied at least three times a day and especially after meals. I believe the dentist should aid in the selection of a tooth brush in order to avoid injury to the gums and teach his patient, when necessary, how to apply it properly. I suggest the use of a tooth paste carrying either mercurophen or metaphen

with or without the addition of arsphenamin as an adjuvant in the treatment of practically all cases. It is true that some commercial dentifrices possess some degree of bactericidal activity, but when these are diluted with water and saliva they are not apt to be sufficiently bactericidal and spirocheticidal to be useful from this standpoint.

I believe that medicated tooth pastes serve a useful purpose in the treatment of all cases and especially those of incipient infection and as a "follow up" after a cure has been effected. The paste should be mildly alkaline in reaction and otherwise acceptable as a dentifrice. A useful prescription is as follows:

Mercurophen or metaphen	0.5 gm.
Tooth paste	100.0 gm.

This gives a 1:200 preparation which appears unnecessarily strong but it is to be remembered that the paste is diluted by saliva and water when used. To this paste may be added 1 gram of arsphenamin but the resulting preparation has a greenish-yellow color not at all compatible with pharmaceutical elegance. So far I have not been able to overcome this objection, although, the addition of 0.1 or 0.2 c.c. of a 1 per cent. solution of basic fuchsin to each 100 grams of the paste improves the color and probably increases slightly its bactericidal activity for gram negative organisms without danger of staining the gums or teeth. The addition of acriflavin also tends to improve the color and aids in increasing bactericidal activity but pastes made up with acriflavin alone in sufficient concentration to be bactericidal when diluted with saliva or water, are apt to be objectionable from the standpoint of staining of the tissues. The same is true of gentian violet and other dyes like brilliant green and crystal violet employed by Bunting and Palmerlee.[85]

Tooth pastes of these kinds can be made agreeable to patients and I believe should be prescribed for all cases during active treatment and as a routine prophylactic against re-infection. They are well borne by the gums and need not be feared from the standpoint of systemic poisoning from swallowing small amounts during application although patients should be informed of their nature and cautioned against swallowing the paste. Both mercurophen and metaphen are slightly less toxic than mercuric chlorid but even were they of the same toxicity, the swallowing of a grain a day would not produce toxic effects and this would correspond to about 10 grams of the paste. It is likely that smaller amounts of mercurophen or metaphen could be used, like 0.1 gm. to 100 grams of paste giving a 1:1000 dilution, but the bactericidal and spirocheticidal effects are less. It is my custom at present to prescribe 0.5 gm. per 100 grams of paste during the active treatment of the disease and to reduce the amount down to 0.1 or 0.2 gm. per 100 grams for routine prophylactic use.

In using these pastes it is well to show the patient how to rub small amounts into the gums by means of the index fingers; the paste is left for at least a minute or two when brushing is done in the usual manner with a brush softened in warm water. As a general rule sufficient saliva is present to give a good lather.

In addition it is sometimes advisable to prescribe a lotion for use several times a day and the following can be recommended:

Mercurophen	0.1 gm.
Peppermint water	100.0 c.c.

These give a 1:1000 solution of mercurophen and bactericidal tests have shown that it is capable *in vitro* of completely sterilizing in one minute an equal part of undiluted saliva in final dilution higher than 1:200,000 or an equal part of a 1:100 dilution of a twenty-four hour broth culture of staphylococci in final dilutions as high or higher than 1:200,000. About a teaspoonful of solution may be diluted with 2 or 3 parts of water, taken into the mouth and left in contact with the gums for several minutes when it should be expectorated and the mouth rinsed with water if the taste is disagreeable. Mercurophen does not form amalgams with metallic fillings of teeth.

Chemotherapy of Cancrum Oris with Arsphenamin and Its Substitutes.—Inasmuch as smears of the secretions of this dreaded infection sometimes show the presence of large numbers of spirochetes and fusiform bacilli, it may be advisable to employ arsphenamin or one of its substitutes in addition to the usual treatment, as suggested by DeSanctis.[86] For this purpose neoarsphenamin may be injected intravenously or sulpharsphenamin intramuscularly in dose of 0.01 gm. per pound of body weight, and it may be advisable to infiltrate the margins of the involved tissues by a series of subcutaneous injections of 1-per cent. solutions of sulpharsphenamin in sterile water in an effort to prevent extension of the infection.

Chemotherapy of Pulmonary Spirochetosis.—While spirochetes are not ordinarily found in the normal bronchial secretions, they may be present in the secretions of some cases of bronchitis, bronchiectasis, pneumonia and lung gangrene, and I wish to discuss the subject, particularly from the standpoint of treatment with arsphenamin and its substitutes, under the general heading of *pulmonary spirochetosis*. Indeed, spirochetes may also invade the pleural cavities with the production of pleural spirochetosis, a case due to *Sp. bronchialis* (Castellani) having been recently reported by Mason.[87] The subject has not received the attention it deserves and it is not at all improbable that cases of acute, subacute and chronic bronchitis are sometimes primarily spirochetic infections, and in bronchiectasis and pulmonary gangrene spirochetes may at least exert an important role as secondary invaders. In some cases of pulmonary consolidations regarded clinically as tuberculous, it is not improbable that spirochetic infection exists and, in gangrene of the lungs following pneumonia, the secondary infection of the involved areas have been known to be spirochetic, with a favorable therapeutic response to the administration of arsphenamin and its substitutes. Doubtless these conditions are frequently overlooked, because the clinical syndromes are not typical and the presence of spirochetes in the secretions overlooked in our search for tubercle bacilli and other bacteria, and because special examinations for spirochetes are not made.

As reported by Rothwell,[88] Greeley,[89] Campbell and Dyas,[90] McNeill[91] and Jackson,[92] the bronchi and lungs may be sometimes infected with the fusiform bacillus and spirochete of Vincent's angina, producing sudden unexplained hemorrhages or lesions that may be clinically and roentgenologically indistinguishable from pulmonary tuberculosis but which heat almost magically under intravenous arsphenamin therapy. Pilot and Davis[93] have recently reported extensive studies in thirty-seven cases of pulmonary abscesses, gangrene and bronchiectasis associated with the fusiform bacilli and spirochetes of Vincent in which pyogens, particularly streptococci, played an important secondary rôle in producing a distinct clinical type of fusospirochete pneumonia. Predisposing lesions about the teeth and gums were usually present suggesting a descending infection; arsenic, particularly neoarsphenamin, exerted a definite therapeutic effect and especially in early cases. In 1907 Castellani[94] described a type of bronchitis due to a spirochete which he believes is a separate species and for which he proposed the name *Sp. bronchialis*. Since then numerous cases have been recorded from different parts of the world, but especially in the tropics, the literature being thoroughly reviewed by Castellani and Chalmers,[95] Fantham,[96] and Bloedorn and Houghton.[97] The disease may manifest itself as an acute attack or it may be chronic or subacute in its nature. In acute cases a chill develops, then fever,

which, according to Castellani, seldom exceeds 39.5° C. This lasts from two to eight days, during which time the patient coughs almost continuously and complains of pains all over the body. The expectoration is scanty, mucopurulent, very seldom containing traces of blood. While arsenicals produce rapid relief, the clinical symptoms may disappear even without treatment. In the subacute type, the general symptoms are similar to those of the acute type, but there may be hemoptysis and bronchitis, with slight crepitations. In the chronic type, the disease may have been slow and insidious in onset, or the patient may give a history of previous acute attacks. When he comes for treatment he has a chronic cough, the sputum is mucopurulent, often blood-streaked, while in many instances there is considerable hemoptysis. There may be moist or dry rôles or consolidation areas. In many cases there is a daily rise of temperature, sometimes reaching as high as 40.5°C.

The disease may be simple or it may be complicated by pneumonia, pulmonary gangrene, empyema, tuberculosis of the lungs, moniliasis, paragonimiasis or rhinitic spirochetosis. While a certain proportion of cases will be found to be free from complications, a large share of them will probably prove to be primarily cases of pulmonary tuberculosis with a complication of bronchospirochetosis. In either instance it is extremely important to examine for these spirochetes the sputum of all persons giving a history of hemoptysis. If the spirochetes are found to be the primary agents of the disease, relief is easily effected by intravenous injections of arsphenamin or neoarsphenamin. If the disease is complicated by some other infection, then spirochetosis can be eliminated, leaving the physician free to treat the primary agent of the disease.

In addition to these three forms of spirochetic bronchitis, Violle[98] has described a type termed *hemorrhagic bronchitis with spirochetes*, in which the secretion of the bronchial mucosa is abundant, nearly a quarter of a liter in twenty-four hours. It is tenacious, stringy, viscous, homogeneous, of a bright rosy color, resembling gooseberry juice. It is sufficient, writes Violle, to have seen it once, to escape the danger of confounding it with tuberculous hemoptysis. The general condition is good, often, indeed, very good; there is no fever; the expectoration contains spirochetes in great abundance. The organisms show an extraordinary polymorphism, and this variation in their shape and dimensions is one of their special characteristics. The prognosis is generally favorable, the bloody expectoration ceasing after ten to fifteen days, with fairly rapid recovery; relapses and chronic bronchitis, however, may follow with periods of exacerbation.

During the World War Nolf and Spehl[99] described eleven cases of another type somewhat different from those described by Castellani and Voille, in which a bronchitis or bronchopneumonia was especially characterized by the fact that the breath and expectoration became fetid rather early in the disease and for which they proposed the name "*fetid spirillar bronchitis*." In addition to the fetid secretions and breath, there was high, remittent fever with no tendency to fall spontaneously, progressive loss of strength, dyspnea on the slightest effort, and more or less cyanosis.

In the United States about twenty-four cases of bronchial spirochetosis have been reported by Kline,[100] Dick,[101] Davis and Pilot[102] and Kline and Blankenhorn.[103] Clinically, some of these were first confused with tuberculosis, abscess, and pneumonia. The sputum in some was jelly-like and blood-tinged, in some hemorrhagic, in some purulent, and in others brown and foul smelling. The disease has occurred somewhat more frequently in males, the patients ranging in age from four to seventy-six years, and recovery has been reported in the majority. Faust[104] has described a series of cases observed in China.

Whether these types of bronchitis and bronchopneumonia are actually caused by a new specific species of spirochetes or by spirochetes from the mouth belonging to the several varieties occurring about the gums and especially described by Noguchi, can not be stated, since our methods for differentiating the spiral organisms are too inadequate for drawing fine distinctions. It is possible that the spirochete and fusiform bacillus of Vincent's angina may produce a bronchitis, as described by Rothwell[105] in 1910; Mellon,[106] Sinclair[107] and others have described cases of whooping-cough, pneumonia and tuberculosis in which spirochetes and fusiform bacilli were found in the sputum. Not infrequently one finds spirochetes in the secretions of chronic bronchitis and bronchiectasis, sometimes associated with fusiform like bacilli, but always in association with enormous numbers of bacteria and especially staphylococci, streptococci and pneumococci, and for this reason the rôle of the spirochetes is usually in doubt and especially if the patient has a spirochetic gingivitis, which suggests this source for the presence of the organisms in the saliva and sputum.

Cases of spirochetal *pulmonary gangrene* and *abscesses* have been recorded by Rona, Kline and Blankenhorn, Fishberg and Kline,[108] Rawson,[109] Spano,[110] and others. It would appear that spirochetes may produce pulmonary consolidations with abscess formation, and especially spirochetes in association with fusiform bacilli from foci about the teeth and gums, a pulmonary type of Vincent's infection; in addition, it is possible that these organisms may gain access to areas of unresolved pneumococcus pneumonia with the production of secondary infection and gangrene.

As previously stated, the presence of spirochetes in the sputum is readily overlooked unless they are kept in mind and especially tested for. The spirochete of Vincent's angina is generally well stained by carbol fuchsin, but may be decolorized in stains for tubercle bacilli and fail to take up the methylene-blue counterstain sufficiently well. It would appear that smears are best stained by the Fontani method, especially by Noguchi's modification of this stain, and that examinations of this kind are called for almost routinely in cases of chronic bronchitis, with or without bronchiectasis, and gangrene. As stated by Nolf, an interesting point is the greater or less abundance of spirochetes in the expectoration. In certain patients they are very abundant. They may be found, so to speak, in every field of the microscope, sometimes several in each field, even in very thinly spread preparations. But it is not always thus; in other instances, the microörganisms are much rarer. One must sometimes examine a large number of fields before finding one. In a general way, one may say that the gravity of the affection is in direct proportion to the number of the spirochetes. In the fatal cases they are as abundant in the bronchial mucus as is the spirochete of Vincent in the detritus which covers the ulceration of the diseased tonsil. It has seemed to him that the more numerous the spirochetes were, the more fetid was the breath and the expectoration, and it was because in his first patient this fetor was very striking and the number of spirochetes great, that he thought immediately of seeking a relation between one and the other, and recognized the possibility of the pathogenic rôle of the spirochetes. On the other hand, when, under the influence of treatment, the disease tends toward recovery, improvement goes hand in hand with the diminution of the spirochetes in the secretion, a diminution which becomes evident even when the general characters of the expectoration, excepting the odor, have as yet shown no change.

The Wassermann reaction has not been reported upon except in a few cases, but insofar as I could determine in this brief review of the literature, the reactions have been uniformly negative.

Practically all of the writers referred to above have reported favorable results from intravenous injections of arsphenamin and neoarsphenamin in the treatment of spirochetic bronchitis. Nolf and Spehl, writing with particular reference to their type of fetid bronchitis, state that treatment must be long to control the disease definitely. If a series of intravenous injections of neoarsphenamin are given in doses of from 0.45 to 0.6 gm., five or six days apart, a change in the clinical picture, rather slow in its development, may usually be made out. The condition, which, up to that time, has grown progressively worse, shows little by little signs of an improvement, which, interestingly, affect first the elements characteristic of the infection. On the one hand, the fetor of the breath and the expectoration becomes less; on the other hand, there is a diminution in number or even a disappearance of the spirochetes under the microscope. During this early period of arsenical treatment, the fever may remain tolerably high, and the local signs in the pulmonary apparatus are little influenced. It may even be that with the continuation of the arsenic treatment, the disappearance of the spirochetes is brought about without amelioration of the general or local condition.

This circumstance is easily explained when one considers that the spirochetes are far from being the only microörganism to be found in the sputum which contains a particularly varied and abundant bacterial flora. These microörganisms probably add their injurious action to that of the spirochete. It would appear that the lesions produced by the spirochetes are severe, and that, invaded by the microörganisms of secondary infection, they present little tendency to heal. This, perhaps, is the most distinctive character of the affection which Nolf and Spehl have described. Even after the patients had been rendered apyretic, and after their previously purulent expectoration had become mucoid and free from spirochetes, they continued to cough, and auscultation revealed signs of an attenuated but persistent bronchitis. The writers have had to have recourse to the combined action of balsamics, exposure to sun, and respiratory gymnastics definitely to overcome these sequelae which always show an extreme obstinacy. In certain cases they have added to intravenous injections of neoarsphenamin, peptone, administered intravenously every two days.

In pulmonary gangrene, Becher,[111] Steff,[112] Reichmann,[113] Molnar,[114] Curshmann[115] and others have reported favorable results from treatment with arsphenamin or neoarsphenamin and it would appear well worth while to include a series of intravenous injections of one of these compounds at intervals of five to seven days. Since neoarsphenamin is less likely to produce toxic reactions, it would appear to be the remedy of choice, although if hemorrhages were occurring, I believe that sulpharsphenamin by intramuscular or epifascial injection would be indicated in order to reduce to a minimum the effects upon capillaries. Becher,[116] Hirsch,[117] Curshman[118] and Albracht[119] have reported favorable results from the administration of arsphenamin in the treatment of some cases of fetid bronchitis and gangrene of the lungs in which no spirochetes were found. Spano states that neoarsphenamin administered twice weekly by intravenous injection has proven especially efficacious in the treatment of fusospirillar lung abscesses, combined if necessary with surgical or pneumothorax treatment.

Chemotherapy of Tropical Ulcer and Other Localized Spirochetic Infections.—Another form of localized spirochetic infection usually associated with fusiform bacilli is *ulcus tropicum*, an infection, usually of the legs, in the form of ulcers which may be occasionally phagedenic, found in all tropical and subtropical regions of Africa, Asia and America but rarely in temperate zones. Smears generally show numerous spirochetes, associated with *B. usiformis*, which some writers believe are *Sp. vincenti* and others think are a separate species, *Sp. schaudinni* (Prowazek). In differential diagnosis, frambesia, syphilis, oriental sore, tuberculosis, blastomycosis, sporotrichosis, acladiosis and mycosis fungoides, must be excluded.

In addition to local treatment, intravenous injections of arsphenamin and neoarsphenamin have been reported by several writers as successful in some cases. Mercury and the iodids are useless.

Spirochetes and fusiform bacilli resembling those found in Vincent's angina may also produce a form of *ulcerative balanitis*, but this infection yields readily to local treatment. A spirochete (*Sp. aboriginalis*) has been found in granuloma inguinale, but it is now regarded as a saphrophyte rather than the primary etiological agent of this disease; arsphenamin and neoarsphenamin have not proved efficacious, whereas intravenous injections of tartar emetic have yielded excellent results (Chapter XXXIII).

Chemotherapy of Weil's Disease (Spirochetosis Icterohemorrhagica) and Yellow Fever.—Weil's disease, infectious jaundice, is now commonly regarded as caused by *Leptospira icterohemorrhagica*, discovered by Inado and Ido[120] in 1915. Studies by Inado and his associates with arsphenamin in the treatment of guinea-pigs experimentally infected with the leptospira have not yielded very encouraging or definite results, although in some animals the blood and liver were apparently sterilized by intravenous injections of single doses of 0.05 to 0.1 gm. per kilogram. (I surmise that these amounts were meant to read 0.005 to 0.01 gm. per kilogram, therefore corresponding to 0.3 to 0.6 gm. for the adult of average weight.) Noguchi[121] also states that experiments conducted by him in 1917 with arsphenamin, neoarsphenamin, atoxyl and arsacetin, yielded negative results; furthermore, Stokes and Ryle[122] and Stokes, Ryle and Tytler[123] report that arsphenamin has been without therapeutic effect in human cases of the disease and for the present, at least, it must be concluded that arsphenamin and its substitutes are without marked effect and of doubtful value in the treatment of this disease.

These negative results with arsphenamin and its substitutes may be regarded as giving support to the opinion that the leptospira group of organ-

isms forms a type of its own and differs from the other pathogenic genera of *Spirochætoidea*. This is supported by the negative results observed in yellow fever, which Noguchi regards as a leptospira infection. As shown by Noguchi, while arsphenamin and neoarsphenamin are highly poisonous in the test-tube for *Leptospira icteroides*, which he believes is the causal organism of yellow fever, yet in guinea-pigs inoculated experimentally and treated by subcutaneous injections of the compounds, it was extremely doubtful that these drugs mitigated the severity of the infection. Arago,[124] however, reviewing the work of DeBeaurepaire and others in South America, believes that neoarsphenamin, in dose of 0.45 to 0.9 gm. by intravenous injection, exerts a curative effect in yellow fever, but at the present time it must be stated that the results are doubtful and that this disease, like Weil's disease, is not favorably influenced by arsphenamin and its substitutes.

While there are no reports available on the use of arsphenamin and neoarsphenamin in the treatment of *dengue* and *pappataci*, it is likely that the results would be of a negative character as in Weil's disease and yellow fever; Strong[125] has reported that arsphenamin is without curative effects in *verruca peruana*, a disease found on the western slopes of the Andes mountains and resembling in some respects a severe type of frambesia.

Chemotherapy of Spirochetic Infections of the Lower Animals.—As stated in Chapter XXIII, marked success has attended the treatment of *fowl spirochetosis* with the organic arsenicals. Even atoxyl and arsacetin have been employed with success, but arsphenamin and neoarsphenamin, in dose of 0.010 to 0.015 gm. per kilogram by intramuscular or intravenous injection, have yielded much better results in experimental infections with *Sp. gallinarum* (*Spiroschaudinnia marchouxi*). In the natural infections of fowl with this spirochete, however, as well as in spirochetosis of ducks and geese (*Sp. anserina* and *Sp. neveuxii*), the results have not been quite as good, because some are quite resistant, although the majority are apparently cured by one to three injections.

Arsphenamin has also been employed with success in diseases of cattle infected with *Sp. theileri* in Africa, of sheep infected with *Sp. ovina* in the Transvaal, of monkeys infected with *Sp. macaci*, etc.

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PART V

CHEMOTHERAPY OF PROTOZOAN AND METAZOAN DISEASES OTHER THAN TRYPANOSOMAL AND SPIROCHETAL DISEASES

CHAPTER XXXII

GENERAL AND SPECIFIC TREATMENT OF THE MALARIAL FEVERS

While systematic and extensive researches in the chemotherapy of protozoan and metazoan diseases have not been made except in the trypanosomiases and spirochetoses, yet several specific remedies are known to exist for other protozoan infections, and notably quinin for the malarial fevers and tartar emetic for the several varieties of cutaneous leishmaniasis. In a way, they do not constitute chemotherapeutic remedies in the sense that arsphenamin and its substitutes are so regarded, because they were not synthesized for these diseases but discovered more or less accidentally by the peoples among whom the diseases existed; but still they fall into the class of chemotherapeutic drugs because of their specific effects upon the microparasites concerned.

Since the discovery of the trypanocidal and spirocheticidal properties of trypan-blue and other dyes, atoxyl, arsacetin, arsenophenylglycin and finally of arsphenamin (salvarsan), it is no wonder that these compounds, as well as quinin and tartar emetic, should have been used in the treatment of other protozoan and metazoan diseases in a more or less blind, "hit or miss" fashion, with the result that a large literature has accumulated upon the use of these drugs in a wide variety of protozoan diseases.

Possibilities of Chemotherapy in Protozoan and Metazoan Diseases.—But the success which has already attended chemotherapeutic investigations in trypanosome and spirochete infections indicates that there is much hope for developing specific remedies for other protozoan diseases, indeed, much more than in the bacterial diseases. In other words, it would appear that the higher the organism in the scale of life the more differentiated its protoplasm becomes, with greater possibilities for the discovery or preparation of chemical agents having a specific parasitropism without injuriously affecting the cells of the infected host. Furthermore, it would appear an easier task to interfere with the life cycle and processes of the protozoa by means of chemical agents sufficiently for their destruction than is the case with the much simpler and more primitive bacteria.

Finally, with many of the protozoa and metazoa infecting mucuous membranes without gaining access to the blood or lymphatic circulations, as in the

case of the intestinal helminths, the problem of chemotherapy would appear to be a simpler matter than attempting to reach and destroy parasites in the tissues, since compounds may be evolved with direct parasiticidal effects and sufficiently non-toxic for the host to permit of administration by ingestion without local corrosive effects or general systemic toxicity through absorption

Difficulties of Chemotherapeutic Research in Protozoan and Metazoan Diseases.—On the other hand however, chemotherapeutic research in the protozoan and metazoan diseases is greatly hampered by our inability to cultivate the parasites or reproduce their diseases in the animals usually employed for laboratory studies. I do not doubt but that if we could successfully produce the human types of the malarial fevers in the lower animals that systematic chemotherapeutic investigations would soon improve treatment with quinin and no one can deny that this is urgently required not only to possibly improve the cinchona alkaloids by synthesizing new compounds but likewise because the tremendous demand for quinin is hardly met by the number of trees available for furnishing the bark.

While, however, numerous attempts to transmit the *Plasmodium malariae* to guinea-pigs, rabbits, monkeys, mice and rats have failed, one of the latest efforts in this direction having been recently made by Bass,[1] yet it is possible, and indeed quite probable, that very useful chemotherapeutic investigations could be conducted with the parasites fo the lower animals, like *P. kochi*, *P. inui*, *P. cynomolgi*, *P. pitheci* or *P. brasilianum* infecting different species of monkeys, *P. canis* of dogs, *P. danilewski* of birds, etc.

Cultivation or propogation of the parasites *in vitro* on artificial media is not essential since parasiticidal activities *in vitro* of chemical agents bears only a broad and general relationship to the far more important question of specific parasitropism *in vivo* in non toxic doses, but yet I believe that future developments in chemotherapy will be based more and more upon fundamental studies of specific combining affinities of chemical agents for the protoplasm of parasites and that much valuable information may be gained by studies *in vitro* with mass cultures of them.

But the essential requisite for chemotherapeutic research is the reproduction at will of the disease under study in the less expensive laboratory animals in order to open up a method for studying the toxicity of new agents for the living parasites in living animals. The ease with which this is accomplished with the trypanosomes and many of the spirochetes accounts in large part for the great success of chemotherapy in these infections. I do not believe that it is necessary to reproduce in the experimental animals all of the pathological lesions exhibited in natural infections of human beings, although, as in the case of syphilis, this is very helpful, since marked tissues changes greatly influence chemotherapeutic activities; but the main requirement is to devise or discover ways and means for producing and maintaining infections in the lower animals with methods for ascertaining the effect or lack of effect or our chemical agent upon the parasites. In the field of protozoan diseases but few attempts of this kind have been made except in malaria.

Owing to the world-wide distribution of the malarial fevers and especially their high incidence in tropical and subtropical countries where chronic infections produce extreme degrees of physical, mental and moral degradation of enormous numbers of individuals, the subject of specific therapy has deservedly commanded a great deal of attention. Indeed, it would appear that the discovery by South American Indians of the curative properties of Peruvian bark for malaria constitutes the first discovery in specific therapeutics and, along with mercury for syphilis, was the only known specific until comparatively recent times. But while there is a popular impression

that quinin in most any form and by most any route of administration is a sure cure for malaria, such is far from being the case and there is much room and urgent need for evolving a superior remedy and improved methods of administration, especially in the treatment of acute subtertian or malignant malaria aestivo-autumnal fever caused by *Plasmodium falciparum* (*Laverania malariae*) and the chronic forms of all varieties.

In many respects the chemotherapy of malaria offers problems quite similar to those later discussed in relation to syphilis. In almost the same manner the matter of the stage of malaria, whether acute, chronic or latent; the sexual and asexual phases of the different varieties of plasmodia; the influence of immunological processes and pathological tissue changes, greatly influence the method of treatment, even though our choice of drugs may be principally limited to the salts of quinin rather than present a broader selection, as is possible in syphilis among the organic arsenicals, mercurials and bismuth compounds.

Of course, an enormous literature has accumulated on the subject of malaria in all of its phases including treatment. It is hardly necessary or profitable even to attempt its review in a treatise of this kind and I shall confine myself to a discussion only of the treatment and especially the specific treatment, with such references to the parasites, their life cycles, pathogenesis, immunity and pathological changes as are directly concerned in these subjects.

Varieties of Plasmodium Malariae in Relation to the Chemotherapy of the Malarial Fevers.—It is now generally accepted that the malarial fevers of human beings are caused by three parasites, namely, *Plasmodium vivax*, producing tertian fever, *Plasmodium malariae*, producing quartan fever and *Plasmodium falciparum* or *Laverania malariae*, producing subtertian fever also known as aestivo-autumnal, malignant or tropical malaria. Additional varieties have been described as pathogenic for man, among the more recent being *Plasmodium ovale* by Stephens,[2] which Huck and Bowden[3] state was recently found in a native of Maryland, but it is likely that this parasite may be a variety of *Plasmodium falciparum*. Many more varieties are known to infect the lower animals, but none of these infect man, and conversely those infecting man do not infect the lower animals, although Mesnil claims to have produced a temporary infection of an orang-utan with *Plasmodium vivax*.

Of the three pathogenic species for man, the first two, namely, *P. vivax* of tertian fever and *P. malariae* of quartan fever, are the most benign and most susceptible to destruction by quinin. This is largely because these parasites circulate in the peripheral blood in which the latter also sporulates, while the former sporulates in the spleen; *P. falciparum* (*Laverania malariae*), on the other hand, sporulates in various of the internal organs and affects the corpuscles so profoundly that they are liable to adhere to the walls of the capillaries and produce profound changes and symptoms by embolism and the local effects of toxins, as coma or paralysis when the brain is involved. Because of the fact that this parasite sporulates in the internal organs and largely in the spleen and bone marrow, it is more difficult to reach and destroy by quinin and especially in chronic infections, after the parasites have produced inflammatory tissue changes.

All three varieties may produce acute and chronic fevers and it hardly requires statement that the acute infections are far more amenable to treatment and even complete cure in the sense of sterilization, than the chronic infections; indeed, we are confronted in the latter by all of the difficulties offered by chronic syphilis and the matter of complete cure becomes doubtful of accomplishment.

P. vivax and *P. malariae* do not tend to localize in any particular internal organ except the spleen, but *P. falciparum*, may involve any or all of the internal organs, producing a variety of clinical types of chronic subtertian or aestivo-autumnal fevers which bid fair to outdo in variety the many clinical types of chronic syphilis. No one has yet raised the question of tissue specificity of strains of plasmodia and especially of *P. falciparum*, and it is not known whether a special strain exists for the brain, producing the meningitic or nervous system localizations on the one hand, with types possessing special tendencies for localiza-

tion in the digestive, circulatory, special sense or other organs, on the other. As in syphilis, I believe the matter of localization of these parasites is largely determined by non-specific factors, among which may be mentioned congenital predisposition and acquired trauma. However that may be, the types of aestivo-autumnal or subtertian fever that may be encountered as a result of localization of the parasites in this or that organ or "system" is bewildering and complex to say the least, and in the malarial districts I would modify Osler's dictum to read: "Know malaria and syphilis in all its manifestations and relations and all other things clinical will be added unto you." Unfortunately, it is just in these multitudinous types of subtertian malaria, and especially chronic malaria, that quinin may fail, although something has been apparently gained in the past few years by combination types of therapy employing methylene-blue and quinin or, better still, arsphenamin or its substitutes with quinin, the arsenicals appearing to increase the ameboid movements of the parasites and tending to rout them out of their tissue strongholds, as it were, into the open circulating blood where they may be more effectively reached and destroyed by quinin.

The Life Cycle of Plasmodium Malariae in Relation to the Chemotherapy of the Malarial Fevers.—But of even greater importance in relation to specific therapy is the matter of the life cycles of the different parasites. The asexual forms and schizonts or undifferentiated sexual forms of all varieties are far more susceptible to quinin than the gametocytes or sexual forms. Indeed, the great problem in the chemotherapy of malaria awaiting solution is the discovery of an agent with greater parasitropism for the sexual forms, that is, for the macrogametocytes and microgametocytes of all species.

When the parasites are first deposited in the tissues by anophelines they are in the form of minute, naked, fusiform bodies called sporozoites, which are very susceptible to quinin and for this reason the drug has proved very efficacious for the medicinal prophylaxis of all types of malaria. These minute parasites now enter erythrocytes, when they are called trophozoites, and develop into larger forms called schizonts; the latter now sporulate or divide into segments producing rosettes of merozoites. These now rupture into the blood stream, each merozoite being a new, naked or free parasite which enters fresh corpuscles to begin again this asexual cycle. It is also possible for them to pass the placenta and enter the fetal circulation, cases of congenital malaria being well known and recently reported again by Forbes,[4] Freire[5] and others, although congenital malaria is much rarer than congenital syphilis, is transmitted only by the mother and never, to the best of my knowledge, by the father in the semen.

As may be surmised, quinin is most effective when the naked parasites are present in the blood or just after they have entered corpuscles, the mature intracorpuscular forms being more resistant. In a general way, the chill of malaria corresponds to the sporulation stage, when the parasites rupture with the discharge of merozoites into the blood stream; the fever stage corresponds to the time when these merozoites are entering fresh corpuscles; the crisis of the attack corresponds to the early or trophozoite stage of intracorpuscular development and the intermittent or afebrile stage, to the time when these young papasites are maturing into the adult forms, shizonts or rosettes. Quinin, therefore, should be available in the blood at the close of the chill to "catch" the young, free or naked merozoites; also during the fever stage to "catch" them as soon as possible after entrance into fresh corpuscles and likewise and especially, during the crisis of the attack, before the intracorpuscular parasites have developed into the mature forms or shizonts. All of this is comparatively simple in uncomplicated tertian and quartan fevers which follow a fairly definite cycle, but in subtertian or aestivo-autumnal fever the cycle may be so irregular as to offer many difficulties and the same is true of double or triple tertians or quartans and especially difficult in mixed infections of any two or all three of these different species of plasmodia.

Furthermore, as stated above, the sexual forms are even more difficult to destroy, and unless a malarial case is promptly and completely cured, these forms are apt to develop in a matter of a few weeks or months. Two forms are recognizable in the blood or organ juices (especially the spleen) known

as the female, or *macrogametocyte*, and male, or *microgametocyte*. When blood containing these is sucked up by an anopheline into her stomach, they undergo morphological changes into bodies called macro- and micro-gametes; these conjugate (zygosis) or fuse into forms called *oökinetes* or *vermicules* and these finally sporulate with the production of numerous *sporozoites* which circulate through the mosquito, finally reaching her salivary glands and proboscis from which they may be deposited into the blood of a human being to begin all over again the asexual and finally, this sexual cycle.

Pathogenesis of Malaria in Relation to Chemotherapy.—Under this heading I refer to the different pathogenic agencies concerned in the production of the malarial paroxysms and the relation of these to chemotherapy.

In the first place, the mere presence of the parasites in the blood may not produce paroxysms of chills, fever and sweat, unless the infection is numerically heavy enough; thus Ross has calculated that in severe malaria about 12 per cent. or approximately 3,000,000,000,000 corpuscles are involved, while if the number of parasites is reduced below 1 for each 100,000 corpuscles—i.e., 250,000,000—they will cause but little disease beyond a gradual enlargement of the spleen and perhaps some anemia and melanosis. Certainly large numbers of parasites can be present in the body and especially in the spleen, without producing much damage, constituting a state designated as latent malaria. As will be shortly discussed, this condition may be due to antibodies or immunological tissue resistance or produced and maintained by the chronic intermittent ingestion of quinin, being quite similar in this respect to the latency of syphilis and equally subject to conversion into active malaria by a reduction in general vitality, trauma or the administration of antimalarial drugs, the latter constituting, as it were, malarial Herxheimer-like reactions.

But very little is known, however, of the poisons or toxins produced by malarial parasites. It is commonly stated that the chills and fever are due to the liberation of a pyrogenetic toxin at the time the parasites sporulate and discharge into the blood; this toxin is also apparently capable of producing some hemolysis or this effect may be due to a second hemolytic poison or malarial hemolysin (hemotoxin) analogous to the hemolysins produced by other protozoa, which I will discuss with more detail later in relation to the nature of malarial hemoglobinuria and blackwater fever. At any rate it would appear that these toxins are excreted by the parasites into the corpuscular hosts followed by liberation into the blood upon the rupture of the infected corpuscles. The pyrogenetic poison is then apparently capable of acting upon non-striped muscle, causing a constriction of capillaries and especially of the skin (goose-flesh of the chill), with stimulation or depression of the cardiac, respiratory and heat regulating centers. This poison, in my opinion, may also alter the corpuscular proteins and especially the hemoglobin to such an extent that the corpuscular fragments act as non-specific protein agents producing in part the chill, fever and sweat, characteristic of non-specific protein or colloidal reactions, so well known nowadays following intravenous injections of vaccines, tissue extracts and certain heavy metals in colloidal suspension. The production of hemolysis by this poison or the second hemolytic toxin, may in turn likewise produce reactions of this kind analogous to the effects following intravenous injections of water, arsphenamin, or other hemolytic agents; and finally, the fragments of corpuscles themselves may produce colloidal shock reactions of chills, fever and sweat exactly analogous to the malarial paroxysm.

In addition to these secreted or exogenous toxins, it is possible that the merozoites and schizonts contain these poisons within their protoplasm as endotoxins and that these may be liberated by the destruction of the parasites by quinin and thereby account for the febrile reactions sometimes observed following quinin administration. In other words, I believe that the malarial paroxysm may be the summation of the effects of the following: (*a*) the liberation of preformed toxins stored in the corpuscles and liberated by sporulation; (*b*) the liberation of endocellular toxins of the same kind following quinin or other disintegration of the parasites; (*c*) the effects of non-specific protein reactions from the transformed corpuscular proteins and (*d*), the effects of colloidal shock reactions due to the corpuscular fragments.

Quinin, however, is not a neutralizer of these toxins or protein-colloidal agents, and just as part of the febrile reaction in syphilis, following the administration of arsphenamin and its substitutes, may be due to the liberation of *Sp. pallida* toxins, so quinin may liberate plasmodial toxins; quinin, however, may, and very probably does, tend to correct or prevent the effects of these plasmodial toxins and colloidal agents upon the cardiac

muscle, respiratory and heat regulating centers as it may in the case of pneumococcus and other bacterial toxins; but the actual neutralization of the plasmodial toxin or toxins, as well as of the protein poisons, requires the operation of the patient's immunological mechanism.

In addition to these acute effects, we may have in chronic malaria the tissue changes produced in the spleen, bone marrow and other organs characterized especially by chronic hyperemia and fibrosis due to the action of these toxins as well as to the inflammatory effects of the malarial pigment and protein poisons. These chronic tissue changes are due entirely to the plasmodia and not to quinin since I have found in my experiments that even the prolonged administration of quinin to rabbits in dose corresponding to one half grain or 0.03 gm. per kilogram of the bisulphate by ingestion daily for three to six months, did not appear to produce any of the tissue changes characteristic of malaria, although intramuscular and especially intravenous injections of large repeated doses were observed to produce profound tissue changes.

Pathology of the Malarial Fevers in Relation to Chemotherapy.—There is nothing in the acute malarial fevers, from the standpoint of pathological tissue changes, that materially influences the question of chemotherapy. Even in chronic malaria the characteristic vascular changes and interstitial fibrosis do not militate against the access of quinin and other plasmodial poisons to nearly the extent that *Sp. pallida* is protected by vascular sclerosis and organized syphilitic exudates. The chief difficulty in the treatment of chronic malaria, and especially of the subtertian or aestivo-autumnal type, is not that drugs fail to reach the parasites, but that it fails to penetrate or destroy them and especially the sexual forms. Indeed, in malaria, the parasites are generally accessible in the capillaries, in sharp contrast to the perivascular and deeper tissue localizations of spirochetes and trypanosomes. When confronted with a patient presenting an enlarged fibrotic spleen or similar involvement of the organs of the nervous, respiratory, digestive, urogenital systems, etc., our first and natural inclination is to ascribe the failure of quinin therapy to the enmeshment of the plasmodia in fibrous tissue and exudates out of reach of the drug. I do not deny that this may occur in part; indeed, histological examination of such organs shows that it does occur, but not to the same extent as in chronic syphilis. In syphilis of the central nervous system and especially in paresis, as well as in cardiovascular and other types of visceral syphilis, it is largely a matter of evolving a spirocheticidal drug capable of escaping from sclerosed vessels and soaking into the parenchyma to reach the spirochetes; in chronic malaria it is rather a matter of evolving a drug capable of destroying the sexual forms of the plasmodia after being brought in contact with them.

Immunity of Malaria in Relation to Chemotherapy.—Many of the immunological aspects of the malarial fevers are of great interest and practical importance. Does natural immunity to the parasites of *Plasmodium malariae* exist among human beings? Does antibody-production or any other immunological change occur in malaria to a therapeutic threshold? Is immunity to malaria transmissible from mother to off-spring? Does one type of malaria afford protection against other types? Do immunological processes ever bring about complete and spontaneous recovery from these infections? Is the latency of malaria an immunological phenomenon? Are there any drugs capable of stimulating or increasing immunological resistance to the plasmodia? Does re-infection and super-infection occur in malaria? These and other equally important questions are concerned

in the immunity of the malarial fevers which may be briefly discussed since they concern the subject of chemotherapy in these diseases.

It is highly probably that natural immunity to *P. vivax, P. malariae* and *P. falciparum* (*Laverania malariae*) is quite rare among human beings. Even in the malarial districts women who have developed a high degree of acquired immunity do not apparently transmit this state to their off-spring and infections among the young are very frequent. Of course, in these districts instances of apparent immunity among adults are not lacking, but these are to be ascribed to acquired immunity as the result of infection rather than to natural resistance. Furthermore, it is to be expected that fortuitous circumstances may account for escape from infection when bitten by infected anophelines. Human beings, however, enjoy an absolute immunity to the malarial plasmodia of the lower animals and *vice versa;* why this is so cannot be stated. There is nothing to indicate that the mechanism is due to antibodies in the blood of human beings for these parasites; rather, in my opinion, it is due to the fact that the human tissues fail to supply a pabulum requirement.

Do human beings ever recover spontaneously from malaria unaided by quinin? It would appear to be well established that some infections may wear themselves out and especially the quartan fevers. This result is apparently due to the production of immunological principles inimical to the parasites, but in the majority of cases, the infection passes into a state of latency rather than complete extinction, which, as in syphilis, may amount to what may be termed "clinical cure" without complete sterilization. At any rate, individuals who elect to "fight off" malaria unaided by quinin have but a poor chance for success in the sense of complete recovery. However, spontaneous recovery may apparently occur in cases of chronic syphilis inoculated with tertian malaria as a form of treatment; but this artificial or inoculation malaria has other features differing from natural infections although the occurrence of spontaneous recoveries indicates that the human may develop a definite immunological resistance to malaria.

Very little is known of antibody-production in malaria. Apparently antitoxins are produced for the pyrogenetic and hemolytic poisons and these are also capable of destroying some of the parasites as indicated by the results of Casagrandi's experiments with pigeons infected with *Hemoproteus* (*Halteridium*). In light and exceptional cases, especially in infections with *P. malariae* of quartan fever, all of the parasites may be destroyed, but the patient may be re-infected subsequently by plasmodia of the same or other species. In other words, the immunity of malaria rarely reaches a state of high perfection, not even as high as is developed in syphilis. At most, the acquired immunity is sufficient for throwing the disease into a state of latency with exceptional cases of complete recovery; but the latency is rather easily broken down by chills, starvation, the onset of another acute disease and the presence of some other chronic ailment, which may often be but slight, so that exacerbations are of frequent occurrence. In the great majority of cases, however, the immunological reaction is sufficient for neutralizing the hemotoxic effects of the malarial poisons, and especially those produced by *P. vivax* of tertian fever, but slightly less so for those produced by *P. malariae* of quartan fever and much less for those elaborated by *P. falciparum* or *Laverania malariae* of malignant or aestivo-autumnal fever. I will, however, discuss this subject shortly with more detail in a consideration of the mechanism of the malarial and quinin hemoglobinurias and the nature of blackwater fever.

Furthermore, even if cure is effected by natural or medicinal means, the immunity is of very short duration, since re-infection may readily occur by plasmodia of the same or other species. Even while an individual is suffering with one type of infection, he may become super-infected with another species of parasite, producing mixed infections. However, the adult natives of malarial districts are certainly more resistant to re-infection and super-infection than immigrants, indicating that the antibodies are somewhat effective; but just as the resistance of syphilis is dependent upon the presence in the tissues of living spirochetes, so it would appear that the resistance of malaria is in reality due to clinically latent infection rather than to congenital transmission of resistance or the retention of antibodies after the complete eradication of the plasmodia from the blood and fixed tissues.

There is little or no hope, therefore, for developing a means for active or passive immunization against malaria, even though we should be eventually successful in cultivating the parasites at will and in mass. Just as in syphilis, the human body cells do not appear capable of producing large amounts of antibody to the parasites in a living state and undoubtedly much less to dead parasites in the form of a vaccine. It is very doubtful if a successful serum therapy can be developed, although the horse or other lower animal may react better than human beings. But at any rate, nothing worth while has been developed so far in malaria in the way of vaccine or serum therapy and our present knowledge of the immunology of the disease gives but little encouragement to investigations in this field.

It is apparent, therefore, that medicinal treatment is the main hope of prophylaxis and recovery in the malarial fevers. But may quinin stimulate antibody-production? May it act upon the antibody-producing tissues in such a way as to increase their response to antigenic stimulation by the proteins of the plasmodia, as some believe that mercury and the organic arsenicals increase antibody-production in syphilis? May the beneficial effects of methylene-blue and arsphenamin in malaria be ascribed to this kind of mechanism? I shall shortly discuss the antiplasmodial mechanism of these drugs in more detail, but here it may be stated that there are no grounds at all for assuming that quinin, methylene-blue and the arsenicals materially aid in the curing of malaria by increasing antibody-production.

Quinin and Other Cinchona Alkaloids in the Chemotherapy of the Malarial Fevers.—The best known and most effective destroyers of all varieties of the malarial plasmodia are the natural alkaloids of Peruvian bark (*Cinchona officinalis*) and their synthetic salts. The curative properties of the bark itself were doubtless known to the natives of Peru before being brought to the attention of the early Spanish explorers of this country about 1600 A.D. In 1683, the Countess of Chinchon, then residing in Peru, was cured of an attack of terian fever by the powdered bark of these trees, called by the natives "quina-quina;" four years later she introduced it into Europe for the treatment of malaria on her estates near Madrid and the tree was subsequently named by Linnaeus in her honor, but who, by the way, misspelled her name. In 1820 Pelletier and Caventou prepared the alkaloid quinin and since then a large number of other alkaloids have been isolated from the bark and additional ones, like hydroquinin and optochin, synthesized from quinin.

Naturally, a good deal of attention has been given to the question of the comparative toxicity and antimalarial properties of these different alkaloids, but the consensus of opinion appears to be that the natural alkaloids are superior to the newer synthesized compounds. Indeed, the number of quinin compounds before the profession is most bewildering and especially in view of the fact that almost all of them have been cham-

pioned by some one as possessing superior properties. One of the latest comparative studies has been made by MacGilchrist,[6] with the general result that of the five natural alkaloids, quinin, quinidin, cinchonin, cinchonidin and quinoidin and the two quinin derivatives, hydroquinin and ethylhydrocuprein (optochin), the relative therapeutic values were determined in the following order, hydroquinin being the most and quinoidin the least effective: (1) hydroquinin hydrochlorid, (2) cinchonin sulphate, (3) quinin sulphate, (4) quinidin sulphate, (5) optochin hydrochlorid, (6) cinchonidin sulphate and (7) quinoidin. For all practical purposes, the first four were found to be of equal value, but if any difference does exist, cinchonin is to be regarded as superior to quinin and quinidin as inferior. As regards unpleasant by-effects of these alkaloids, buzzing in the ears was most frequently associated with quinin and quinidin; amblyopia, with quinin and cinchonin; diarrhea, with cinchonin if administered for over a week; nausea, with quinoidin. Cinchonidin, even in large doses, caused no unpleasantness of any kind.

It is not surprising that a host of other vegetable compounds should have been advocated from time to time to substitute for quinin in the treatment of malaria or to act as adjuvants; the sheet anchors of treatment are quinin, cinchonin and their salts. Ethylhydrocuprein (*optochin*), and particularly its soluble hydrochlorid, being found more bactericidal for the pneumococcus than quinin and other natural alkaloids, has been advocated by Izar[7] and others for the treatment of malaria, but is now known to be actually less effective than quinin and more toxic; the same is true of isoamylhydrocuprein (*eukupin*) and isoocytylhydrocuprein (*vuzin*).

Absorption and Elimination of the Cinchona Alkaloids in Relation to the Chemotherapy of the Malarial Fevers.—It is commonly believed that quinin bisulphate and other salts of quinin and cinchonin are largely absorbed from the stomach, since quinin may appear in the urine as early as fifteen minutes after ingestion. MacGilchrist claims that absorption is largely from the small intestine, but it would appear that the acidity of the stomach facilitates absorption to a greater degree than the alkalinity of the small intestines. At any rate, absorption after ingestion is ordinarily quite rapid and usually sufficient for the treatment of the majority of cases of malaria.

When quinin and cinchonin reach the stomach, it is probable that minute portions are converted by the hydrochloric and organic acids into quinicin (quinotoxin) and cinchonicin (cinchonotoxin), but these substances are not nearly as toxic as first surmised and the myth of incompatibility of quinin with acids under these conditions has been long since exploded. Indeed, one of the drawbacks to the oral administration of quinin and especially in the treatment of chronic malaria, is the frequent occurrence of anacidity or hypo-acidity of the gastric juice which hinders absorption of the drug and requires the administration of a mineral acid like hydrochloric or sulphuric acids. Furthermore, regurgitation of the alkaline duodenal contents and especially in "bilious remittent fever," tends to neutralize the stomach acidity and greatly interferes with the absorption of quinin. Failure of quinin administered by ingestion to control malaria is frequently due to hypo-acidity or regurgitation of bile and it is sometimes necessary to first cleanse the stomach preliminary to the administration of the drug and to accompany its administration with a diluted acid.

The salts of quinin and cinchonin are also administered by intramuscular and intravenous injection and especially in cases of severe malaria of all types associated with vomiting or coma interfering with administration and absorption by the oral route. These methods have been particularly serviceable in the treatment of aestivo-autumnal or malignant malaria and S. Solis-Cohen[8] has especially advocated deep intramuscular injections of the double salt known as quinin and urea hydrochlorid in dose of one gram (15 grains) dissolved in a syringeful of sterile hot water. As will be shortly discussed, however, quinin produces a considerable degree of local irritation and intravenous injections must be given with great care on account of the production of dangerous reactions. It is certain that intramuscular injections are to be preferred to intravenous injections when the oral route cannot be employed, but it is possible to give intravenous injections with considerable safety if the drug is highly diluted and slowly injected, and this route is sometimes required for the prompt checking of severe malarial infections and especially aestivo-autumnal, subtertian, tropical or malignant malaria.

After the absorption of quinin, about 60 to 90 per cent. is destroyed in the body and the balance is eliminated in the urine; none or but traces have been found in the feces and other excreta. Independently of the dose or route of administration the elimination of quinin in the urine ordinarily begins in fifteen to thirty minutes, reaches the maximum in

four to ten hours, and is practically completed in twenty-four hours, although traces may be found for several days. In hypo-acid or anacid states and in bilious vomiting, however, absorption is delayed or does not occur and for this reason, when quinin is administered by stomach, it is sometimes useful and practical to apply a test for quinin in the urine as a means for determining whether or not absorption is occurring. In severe nephritis and cardiac decompensation elimination may be delayed. Sometimes it is found that patients do not take the quinin ordered and the following urinary test is advocated by Castellani and Chambers as a means for determining whether or not absorption is taking place: (*a*) Place 2 c.c. of *filtered* and *clear* urine in a clean test-tube. (*b*) Add 0.5 c.c. of *Tanret's reagent* (3 gm. potassium iodid, 1 gm. bichlorid mercury, 20 c.c. of glacial acetic acid and distilled water to make 60 c.c.). In the presence of quinin or albumin, an *opalescence* or *white cloud* appears. (*c*) Add a few drops of absolute alcohol, when the precipitate, if due to quinin will disappear, while that caused by albumin will remain. The reaction appears in about two hours after taking a dose of quinin and persists for twenty-four hours after a dose of 5 grains, and for forty-eight hours after a dose of 20 grains of the drug.

When quinin is administered by ingestion, the concentration in the blood remains quite constant for about twenty-four hours, as shown by Hartman and Zila;[9] after intravenous injection, the concentration sinks rapidly at first and then more slowly. In the blood it would appear, according to Baldoni,[10] that most is contained in the erythrocytes, but the brain, liver and spleen have been found by Baur and his colleagues[11] to contain more than the blood. After intramuscular and intravenous injection, traces may be found in the bile and saliva, but most is eliminated in the urine. This tendency for the rapid destruction and elimination of quinin adds to the difficulties in treatment and especially of chronic malaria when the presence of the drug is required over a long period of time; on the other hand, however, it greatly reduces the chances of producing toxic tissue reactions and especially of the liver and kidneys, which may occur after the prolonged administration of mercury, arsphenamin, etc.

Toxicology of the Cinchona Alkaloids in Relation to the Chemotherapy of the Malarial Fevers; Allergic Hypersensitiveness to Quinin.—The untoward or toxic effects of quinin, cinchonin, and other alkaloids of Peruvian bark are so well known under the term "cinchonism," that they scarcely required description in this treatise. The early symptoms are headache, tinnitus, and blurred vision doubtless due to vascular disturbances in the vessels of the meninges, eye and ear, while in severe or prolonged poisoning, the vasculoconstriction producing ischemia of the retina and ear may be followed by atropic changes in the cells of the retina, optic nerve and spiral ganglion, resulting in blindness and deafness, at first partial, later complete. Very large doses may produce difficulty of speech, confusion of ideas, delirium, coma and respiratory failure due to paralysis of the center, the latter being the usual cause of death. Susceptibility to quinin and cinchonin varies among different human beings; as a general rule, individuals with malaria and pneumonia can stand much larger doses without signs or symptoms of cinchonism than normal, healthy persons. This is analogous to the increased tolerance of syphilitics for mercury and the iodids. On the other hand, individuals may be hypersusceptible to quinin, although true allergic hypersensitiveness is so uncommon that the subject hardly requires the amount of discussion devoted to it. However, it is well for individuals about to pass into malarial districts to determine their sensitiveness to quinin by taking one or two grains to determine tolerance in case it is advisable to take quinin for prophylactic purposes or if the drug would be required should they unfortunately contract the disease.

In hypersensitive individuals the swallowing of even traces of quinin may produce the most marked effects of cinchonism including a variety of scarlatinal, urticarial, purpuric and other rashes. My colleague, Dr. Boerner, who is hypersusceptible to quinin, has

described a skin test[12] for the condition consisting of the application to abrasions of the skin of a drop of 1:10 to 1:1000 solutions of the bisulphate, a marked urticarial reaction occurring in a few minutes. O'Malley and Richey[13] have also described a similar skin reaction to quinin and, curiously, reactions did not occur with cinchonin or cinchonidin nor were the individuals clinically susceptible to these closely allied compounds, suggesting that, in cases of quinin hypersensitiveness, it may be possible to administer one of the salts of cinchonin.

These instances of allergic hypersensitiveness are invariably congenital or natural allergies; I do not know of any of acquired sensitization. Intolerance for quinin, however, may be acquired as a result of prolonged administration, but this is much less likely to occur than intolerance for mercury and the organic arsenicals, since so little quinin is stored up in the body. In some cases of this kind it is possible to administer cinchonin without ill effects, but if not, and if quinin is required for the treatment of a malarial infection, it is worth while to attempt densensitization by the methods employed by Heran and Saint Girons and O'Malley and Richey.

It is stated that the fatal dose of quinin is about 8 grams for an adult (approximately 130 grains), but a dose of 30 grams of the sulphat has been recovered from, probably because this salt is but feebly soluble and much may not have been absorbed. Cohen, Heist and I[14] have found that the maximal tolerated (10 days) doses of several quinin preparations for rabbits and mice were approximately 0.02 to 0.06 gm. by intravenous and 0.03 to 0.08 gm. per kilo by intramuscular injection. Toxicity by intravenous injection is greatly influenced by the degree of concentration of the solutions and rate of injection, but if the average maximum tolerated dose of the dihydrochlorid is taken as approximately 0.05 gm. per kilogram in 5 c.c. of sterile water (1:100), it is found that this is equivalent to 3.5 grams for a 70 kilogram adult, so that the intravenous injection of 1 gram (15 grains) is well within the limits of safety for most individuals. It is frequently advised to give this amount in 10 c.c. of water by syringe injection (1:10), but I believe it is much better to give it in 100 c.c. of sterile normal saline solution by gravity injection in order to minimize the chances of producing toxic reactions. Furthermore, in the type of case requiring intravenous injections, the saline infusion alone is of therapeutic value.

All of the salts of quinin and cinchonin injected intramuscularly and subcutaneously produce varying degrees of local irritation; in the rabbit, I have observed necrosis of muscle cells, polymorphonuclear leucocytic infiltrations, hyperemia and hemorrhages. Some salts like quinin and urea hydrochlorid and quinin with urethan are much less painful than the bisulphat, but in a general way the degree of local irritation varies according to the percentage of quinin or cinchonin in the molecule. Deep intramuscular injections are much less painful than subcutaneous injections; indeed, the latter should not be employed at all and in giving the former, due care should be exercised against depositing any of the drug near large nerves or in the subcutaneous tissues. According to my limited experience, epifacial injections described in Chapter LX are better borne than intramuscular injections and are to be preferred for ambulatory patients.

Since quinin and cinchonin do not tend to accumulate in the tissues, prolonged administration is not usually followed by any evidences of nephritis, hepatitis or other tissue reactions, although gastro-intestinal changes may occur. Cornwall[15] states that intramuscular and intravenous injections of quinin to rabbits over long periods of time may produce some damage of the epithelium of the kidneys and suprarenal glands, with increased disintegration of erythrocytes in the spleen. I have observed that intramuscular and intravenous injections of rabbits with quinin dihydrochlorid, in dose of 2 grams per 60 kilograms of weight every 5 days for twelve to thirty-six injections, sometimes produced nephritis and marked vascular changes; also passive hyperemia and cloudy swelling of the liver and hemosiderosis of the spleen, but these changes were usually slight and not apparent at all in some animals. Larger doses, however, produced more intense changes of the same general character along with hyperemia and perivascular round cell infiltrations of the meninges. I have the impression, however, that rabbits are less susceptible to the vascular changes of cinchonism than human beings; at least this appears to be true of the retinal vessels insofar as one may determine by opthalmoscopic examinations of the eye-grounds, as already discussed in Chapter VI in refernce to the toxicity of ethyl-hydrocuprein hydrochlorid.

Of additional interest in connection with the toxicity of quinin and especially in relation to intravenous injections, are its hemolytic properties, but this subject may be now discussed in relation to quinin and malarial hemoglobinurias and the etiology of blackwater fever.

Malarial and Quinin Hemoglobinurias and the Nature of Blackwater Fever in Relation to Chemotherapy.—It is well known that in exceptional cases of chronic malaria, the administration of quinin, even in single small

doses by ingestion, may produce an acute fever characterized by hemoglobinemia and hemoglobinuria. This condition has been generally designated as "quinin hemoglobinuria," but I am confident that it is a misnomer and that the condition is not to be ascribed to quinin itself but rather to the infection.

Hemoglobinemia, and especially hemoglobinuria, sometimes occurs in malarial infections due to *P. falciparum* (aestivo-autumnal) and exceptionally in fever due to *P. malariae* (quartan) independently of quinin; this condition is designated as "malarial hemoglobinuria," and is usually relieved by the administration of quinin.

Hemoglobinemia and hemoglobinuria, associated with great blood destruction, jaundice, fever, chills, headache, vomiting, delirum, etc., sometimes develops in the malarial districts and is known as "blackwater fever," "bilious hemoglobinuric fever," "bilious remittent fever," "malignant bilious fever," etc. The etiology is obscure; some believe it is due to malaria; others and especially Stephens, believe it is due to the effects of chronic malaria and quinin, and others still, like Castelleni and Chambers, that it is due to some unknown agent which may be a parasite transmitted by the bites of an unknown arthropod. Undoubtedly the majority of cases show plasmodia in the blood and these are benefited by quinin; in most instances *P. falciparum* has been found, but *P. vivax* or *P. malariae* may be present. Stevens and also Thomson[15a] believe that the disease is undoubtedly due to repeated intense infection with *Plasmodium falciparum*—usually over a prolonged period of several months or years and that it should not only be controlled, but absolutely abolished, by anti-malaria measures.

All three conditions, therefore, are characterized by increased blood destruction; in one, this is ascribed to quinin; in another, to plasmodia and in the third, to malaria and quinin or to an unknown agent.

In the first place, is quinin hemolytic? If so, are the erythrocytes of chronic malarial cases more susceptible to the hemolytic action of quinin than the corpuscles of normal healthy subjects? In 1909, Barratt and Yorke demonstrated that quinin in alkaloidal form and as the dihydrochlorid were hemolytic *in vitro*, but expressed the opinion that it was not possible to place enough in the blood stream to produce hemolysis, because of the toxicity of the drug. Others have stated that while quinin was not directly hemolytic *in vivo* that it may be indirectly hemolytic by other ways, as, for example, by lowering the osmotic tension of the plasma or in some way modifying the response to other hemolytic agents. Stephens and Christopher found that 0.001 to 0.00082 gm. of quinin hemolyzed 1 c.c. of a suspension of normal human cells at 37°C. and Castellani and Chambers believe that quinin hemoglobinuria is due to hemolysis of erythrocytes *in vivo* by the drug. I cannot agree, however, with this statement and in my opinion the primary etiological factor in so-called quinin hemoglobinuria is really a malarial infection.

In my experiments various salts of quinin were found to be slightly hemolytic for washed human corpuscles *in vitro*, but this hemolytic effect was greatly inhibited by plasma, just as the hemolytic effects of arsphenamin *in vitro* are inhibited by plasma and serum. For example, in tests employing 1 c.c. of varying dilutions of drugs in saline with 1 c.c. of 1-per cent. suspensions of washed cells or 0.2 c.c. of whole blood, the highest dilutions showing hemolysis after an incubation of one hour at 38°C. were as follows: 1:300 to 1:1800 with washed blood cells and only 1:100 to 1:300 with unwashed cells.

The corpuscles of one chronic case of malaria (*P. falciparum*) yielded identical results. The intravenous injection of rabbits with doses of the bisulphate and dihydrochlorid as high as 0.020 gm. per kilogram, corresponding to 1.5 gm. for the human adult of average weight, has never produced hemoglobinemia or hemoglobinuria, although among some rabbits treated with repeated intravenous and intramuscular injections of these compounds in dose of 0.01 gm. per kilogram, the urine has shown the presence of erythrocytes and albumin, and the kidneys the presence of acute nephritis in which the glomerular tufts were hemorrhagic with collections of erythrocytes in the tubules. In other words, while salts of quinin are feebly hemolytic in whole blood, it is not possible to introduce enough into the circulation of living rabbits to produce hemoglobinemia or hemoglobinuria, unless a toxic nephritis is produced with the passage of blood into the urine, as in other types of kidney injury. I believe the same conditions pertain to human beings and that it may be

stated that quinin itself in the doses ordinarily administered does not produce hemoglobinemia or hemoglobinuria.

On the other hand, the malarial plasmodia and especially *P. falciparum*, may produce some profound change increasing blood destruction. This does not appear to be in the nature of an increased fragility of the erythrocytes; at least they are not of increased susceptibility to the hemolytic activity of quinin. Rather, I believe the condition is due to some alteration of the spleen and bone marrow caused by the plasmodia and especially some change in the spleen whereby the physiological activity of this organ in blood destruction is greatly augmented to reach pathological proportions. At the same time, some profound change has occurred in the blood either in the way of a decrease of antibody counteracting the activity of the plasmodial hemotoxin or some chemical change of unknown character associated with the presence of free hemoglobin or bile and counteracted by calcium, since the administration of calcium lactate by mouth or calcium chlorid intravenously are known to afford relief. Quinin hemoglobinuria, therefore, is a misnomer and whenever the administration of this drug is followed by the presence of hemoglobin or corpuscles in the urine, I believe it is because the drug has stimulated foci of latent plasmodial infection in a manner analagous to the Herxheimer reaction of syphilis. In my opinion, therefore, quinin hemoglobinuria, malarial hemoglobinuria and blackwater fever are all due to the one essential primary etiological agent, namely, the malarial plasmodia. These parasites are responsible for the change in the spleen and other organs concerned in blood destruction producing an exaggeration of this process and resulting in the production of secondary changes in the blood, especially the presence of unchanged hemoglobin and bile, responsible for an alteration in the coagulation and bleeding time with an increased tendency for hemorrhage. Since the most severe chronic infections are due to *P. falciparum*, it is to be expected that these conditions will occur most frequently in aestivo-autumnal fever, but that they may also occur in chronic tertian and quartan fevers. While quinin may be expected to be most capable of stimulating latent chronic malarial infections, I would not be surprised to learn that other agents may produce the same thing and especially intravenous injections of arsphenamin and neoarsphenamin.

Why these changes occur in some cases and not in others cannot be stated; nor why blackwater fever and its milder form, malarial hemoglobinuria, affect only some cases or are especially prevalent in certain districts. In my opinion, these malarial hemoglobinurias are essentially similar to paroxysmal hemoglobinurias, which we now know are frequently due to *Sp. pallida*, but doubtless may be caused by chronic bacterial infections in which the essential change is due to some disturbance of the organs concerned in blood destruction with secondary chemical changes in the blood.

In relation to treatment, I believe that the administration of quinin is indicated in all cases, although in quinin hemoglobinuria, which, as stated above, I believe is really a malarial Herxheimer reaction, and in blackwater fever, which, I believe, is an acute severe type of malarial hemoglobinuria or Herxheimer reaction in case the attack is initiated by the administration of quinin, it may be necessary to administer calcium to check the hemorrhagic diathesis while the infection is being attacked by quinin. But this subject will be shortly discussed with more detail.

Mechanism of the Plasmodicidal Activity of Quinin in the Treatment of the Malarial Fevers.—How do quinin and cinchonin destroy the malarial plasmodia? This is a subject of fundamental importance and interest not

only in relation to the treatment of the malarial fevers, but also in relation to the science of chemotherapy in general.

Does quinin cure malaria by a direct toxic action on the protoplasm of the parasites? Unquestionably quinin is a poison for the malarial protozoa, just as it is toxic for the protoplasm of other cells possessing ameboid movement, as the various infusoria, leukocytes, spermatozoa, etc. It is stated that *in vitro* 1: 10,000 solutions are capable of immediately arresting the movements of the parasites followed by such morphological changes as indicate their death. Bass and Johns have shown that quinin inhibits and destroys the plasmodia in cultures and it has been calculated that a 1: 20,000 dilution in the circulating blood is sufficient for the destruction of trophozoites and other asexual forms of the parasites. At least a dose of 0.2 gm. (3 grams) per 70 kilograms (145 pounds) is capable of ridding the blood temporarily of the early asexual forms of plasmodia and if this dose were absorbed into 4000 c.c. of blood the dilution would be 1: 20,000. In practice, however, several doses of this would follow at intervals of three or four hours so that the concentration would be higher in most instances, but it is well known that 2 to 5 grains of quinin per day is sufficient for prophylactic purposes, that is, capable of destroying the sporozoites deposited in the skin by the bites of infected anophelines. I believe, therefore, that we may logically conclude that quinin may cure malaria by a direct parasitropic effect upon the plasmodia.

But may quinin first undergo some change or unite with some constituent of the blood to form a more toxic substance which is the actual plasmodocidal agent, just as arsphenamin is believed to be first converted into an oxid of enhanced spirocheticidal and trypanocidal properties? To the best of my knowledge there is no evidence to indicate the operation of a mechanism of this kind.

May quinin destroy the plasmodia, by stimulating the production of antiplasmodial substances by the body cells in the nature of antibodies in a manner analogous to the production of antispirocheticidal substances by arsphenamin and its substitutes, which some believe is the mechanism involved in syphilis? To the best of my knowledge there are no data to indicate a mechanism of this kind, although systematic researches in malaria have not been made largely because of the impossibility of securing adequate numbers of the parasites for studies of this kind. But available data indicate that quinin does not increase the production of the antihemolysins for the malarial hemotoxin and I personally doubt if quinin cures malaria by any mechanism of this kind.

Bass and Johns have observed that normal serum is toxic for the plasmodia *in vitro* and have suggested that quinin destroys the parasites by increasing the permeability of erythrocytes for toxic constituents of the plasma. This interesting theory requires confirmation and a mechanism of this kind may, indeed, exert some rôle in the antiplasmodial properties of quinin, but the drug is apparently capable of destroying the young asexual parasites free in the plasma which rather indicates a direct plasmodocidal effect rather than a mechanism increasing the normal toxicity of the plasma.

It is also probable that quinin may reduce the oxidases of the plasma and that this may interfere with the life processes of the plasmodia, but so far there is no direct evidence of this mechanism playing any important rôle in the plasmocidal activity of quinin, although, as discussed in Chapter XXIII, it is believed that the trypanocidal and spirocheticidal properties of the organic arsenicals and the protoplasmic toxicity of arsenic in general may be due to an interference with the processes of oxidation and reduction, that is, the so-called internal respiration of cells.

Whether quinin is less effective against the schizonts and merozoites or full grown intracorpuscular parasites of all types and especially weak in parasitocidal activity for the sexual forms or gametocytes of all types, cannot be stated. It is believed by some that the gametocytes are formed only when the parasites are placed in adverse conditions, as in the presence of small subcurative amounts of quinin, and that they correspond to the spore stage of some of the bacteria as an expression of self-preservation; but be this as it may, the fact remains that these sexual forms are far more resistant to destruction directly or indirectly by quinin than the asexual forms.

Quinin Fastness in Relation to the Prophylaxis and Chemotherapy of Malaria.—Furthermore, it is well established that the malarial plasmodia and especially *P. falciparum* may occasionally acquire varying degrees of resistance or fastness to the plasmodocidal effects of quinin and it is believed that this may account for the difficulties encountered in the treatment of some cases of chronic malaria and especially of aestivo-autumnal fever. The evidence in favor of the acquisition of this state, however, is almost entirely clinical. But there are no reasons for doubting that plasmodia may acquire a resistance to quinin, just as trypanosomes have been rendered resistant to some of the dyes and organic arsenicals, although resistance to quinin in any given case of malaria may be due rather to the presence of sexual forms (gametocytes) of the parasites in the blood and tissues, which are known to be resistant to quinin, without actual acquired quinin-fastness. Moreover in not a few cases of chronic malaria resistant to quinin it has been found that this state was not due to an acquired fastness of the parasites, but rather to the fact that the patient was not actually swallowing the drug or because of gastric disturbances with or without vomiting which interfered with absorption. Indeed, when these factors have been adequately controlled, the number of cases of true quinin resistance have been greatly reduced and confined almost entirely to *P. falciparum* (aestivo-autumnal) infections. One of the most recent experiences showing the importance of first ruling out the matter of absorption before concluding that "quinin-fastness" existed, have been recently reviewed by Fletcher:[16] In 1917, reports began to appear that English soldiers in the tropics were being attacked by malaria that quinin would not cure. Pratt-Johnson and Gilchrist found that relapses were reported in 23 per cent. of 18,000 soldiers in Africa, while quinin was being administered Phear reported in Macedonia that quinin was ineffective in malaria that was complicated by dysentery. Willcox in Mesopotamia encountered cases that did not improve under quinin by mouth for ten days. Mackie found quinin ineffective in malaria in northern Persia, and the accumulation of invalids in Saloniki was constantly increasing. Such contradiction and disappointment concerning quinin led to an extensive experimental and clinical inquiry into the treatment of malaria at the Liverpool School of Tropical Medicine, the results of which, unfortunately, did not entirely restore confidence in quinin.

Many soldiers were sent back to England from Macedonia in 1918, and Sir Ronald Ross arranged to treat the malaria patients in special wards at Southampton. Fletcher states that 1150 patients were admitted to these wards in twelve months, and that on landing, parasites were found in the peripheral blood in 487. Although many had documentary evidence of treatment with quinin in various hospitals, they were placed on 10 grains of quinin by mouth twice a day. There was strict supervision of these patients, and special interest in relapses that might occur. In every instance the routine treatment prescribed at Southampton caused both fever and parasites to disappear.

Fletcher emphasizes the Southampton experience by a report of his observations in the Federated Malay States since the war. The patients seen there were little more than skeletons, and dysentery was a complication in 53 per cent. Forty-four difficult cases in which quinin had been disappointing were selected, and 10 grains of quinin by mouth twice a day was given for four weeks. The drug was placed in the patient's mouth by the physician, and, after it had been swallowed, the mouth was inspected. Not one of these coolies in Kuala Lumpar failed to improve. There was in one a small number of parasites that persisted in the blood in spite of quinin, but in every other case, both fever and parasites disappeared. Dysentery did not make any difference. When quinin was actually swallowed and retained, malaria in dysentery patients was as easily controlled as in patients who did not have dysentery. The conclusion of this study was the same as among soldiers in Southampton, namely, that the so-called resistance to quinin vanished when the quinin was actually swallowed and retained.

In general, Fletcher's report is a plea for the restoration of faith in quinin in malaria, and for its oral administration. He does not say that an absolute resistance to quinin is impossible, but in a wide experience he had not seen one case of actual resistance.

However, actual quinin resistance may be apparently acquired and especially in *P. falciparum* infections, but the mechanism involved is still obscure. At least, the Ehrlich theory of chemoreceptoric atrophy would appear to be insufficient and unsatisfactory, as already discussed in earlier chapters. The parasites are apparently capable of immunizing themselves against the effects of quinin, but just how they do this is unknown. It is of considerable practical value and theoretical interest to note, however, that parasites resistant to the salts of quinin may be susceptible or vulnerable to cinchonin or other alkaloids of Peruvian bark.

Quinin-fastness also bears an important relation to the prophylaxis of malaria in this manner, that the taking of quinin by chronic cases may result in a state of fastness of the parasites which may be capable of transmitting this state to new generations after undergoing the sexual cycle in the body of anophelines. If this were possible, it may be that in malarial districts, quinin-resistant strains of the various plasmodia are propogated. We at least know that trypanosomes rendered resistant to the dyes and arsenicals are capable of transmitting this state indefinitely, but I am not cognizant of any experiments showing whether a resistant strain of *T. gambiensi*, for example, is capable of transmitting this quality after passage through the tse-tse fly or whether quinin-resistant strains of malarial plasmodia are capable of maintaining and transmitting this quality after passage through anophelines. It is entirely likely, however, that such is possible and if so, the importance of protecting the chronic malarial and possibly quinin-resistant cases against mosquitoes is indeed a doubly important matter and worthy of investigation.

Methylene-blue in the Chemotherapy of the Malarial Fevers.—This dye (tetramethyl-thionin hydrochlorid) was introduced by Ehrlich and Guttman for the treatment of malaria on the basis of its selective coloring properties and especially for the treatment of those cases to whom adequate doses of quinin could not be given. Especially good results were claimed in *P. malariae* (quartan) fevers, although some effects were also noted in *P. vivax* (tertian) and *P. falciparum* (aesitivo-autumnal) fevers. Since then, a rather large literature has accumulated upon the subject and with the discovery of the trypanocidal activities of some of the dyes, interest was greatly renewed, but recent studies in malaria with trypaflavin and tryparasan by Kalberlah and Schlossberger[18] and others have shown that these new synthetic dyes are lacking in antimalarial properties.

Methylene-blue, however, in dose of one gram (15 grains) per day by ingestion, is well borne and appears to be worthy of trial in the treatment of chronic malaria along with the administration of quinin and especially in those cases in whom the plasmodia appear to have acquired a fastness or resistance to these compounds; as stated by Reitter,[19] methylene-blue alone is ineffective, but is frequently of aid when given with quinin in the treatment of rebellious cases and especially in aestivo-autumnal fever. Just how or why this should be so I am unable to state. The dye itself is but feebly plasmocidal and probably owes its helpful effects in malaria to an interference with the internal respiration of the parasites by processes of alternate oxidation and reduction. It is likely that quinin owes part of its plasmocidal activities to an interference with the oxidases of plasma and I surmise that the two compounds act synergistically in this manner and thereby aid each other in the destruction of crescents and other quinin-resistant types of the plasmodia.

Arsphenamin and Other Arsenicals in the Chemotherapy of the Malarial Fevers.—With the discovery of the trypanocidal and spirocheticidal properties of atoxyl, arsacetin, arsenophenylglycin and finally of arsphenamin and

neoarsphenamin, it is no wonder that these compounds should have received early trial in the treatment of the malaria fevers and a rather large literature has accumulated upon the use of two latter compounds. It is now well known that the administration of arsphenamin or neoarsphenamin may stimulate cases of chronic latent malaria into activity sometimes with fatal results, as reported by Huck and Bowden,[20] Sklarz,[21] Bernard,[22] Milian,[23] Goresco and Popesco[24] and others. In some cases this has aided in finding the organisms in the blood and establishing the diagnosis.

Arsphenamin was apparently first used by Iversen and Nocht in the treatment of malaria in 1911 and since 1912 a rather large literature has accumulated on the subject with conflicting opinions of its value in the treatment of the disease. Among the early reports, all of which were favorable, may be mentioned those by Werner,[25] Tuschinsky,[26] Ivananoff,[27] Bogan,[28] Reed[29] and Goldthwaite.[30] Falconer Anderson[31] observed good results with arsphenamin and neoarsphenamin along with quinin in the treatment of tertian and quartan fevers, but not in aestivo-autumnal fever; quinin and galyl also yielded good results. Stephens, Yorke and Blacklock,[32] Stott,[33] Neff[34] Manson,[35] Teubern,[36] D'Esterre,[37] Surbek,[38] Bohme[39] and others have reported that arsphenamin and neoarsphenamin are sometimes useful adjuvants to quinin and especially in the treatment of chronic quartan and tertian infections. Marchouix[39a] found the internal administration of *stovarsol* efficacious in tertian malaria in dose of 1 gram several times a day.

However, others claim that arsphenamin and its substitutes are without beneficial effects and may even prove harmful. Thus, Kopanaus,[40] in 1911, although observing some beneficial results in the treatment of tertian fever in man from the administration of arsphenamin, made a very thorough stuy of its effect on proteosoma, an organism producing bird malaria, and found that the drug, in large and small doses, actually lowered the resistance of the birds to the organism without being in itself curative. Kalberla and Schlossberger[41] did not observe any direct plasmodicidal effects of arsphenamin, neoarsphenamin or silver arsphenamin; Stephens[42] states that neoarsphenamin in conjunction with quinin is no more effective than quinin alone in aestivo-autumnal fever and Worner,[43] while considering neoarsphenamin useful for general treatment and for correcting the anemia, states that it is of no value for the treatment of gametocyte carriers.

These clinical reports indicate, therefore, that arsphenamin, neoarsphenamin and possibly stovarsol may possess a slight degree of parasitropism for the plasmodia of the malarial fevers of man and especially for *P. malariae* (quartan) and *P. vivax* (tertian), but these direct destructive effects are very much less than exhibited by quinin. For the sexual organisms or gametocytes of all three species, these compounds are ineffective, but quinin is likewise of greatly reduced parasitotropic effects for these. In acute early malaria, therefore, arsphenamin and neoarsphenamin alone are very much less effective than the salts of quinin and should not be given unless the latter compounds cannot be administered on account of allergic hypersensitiveness. In chronic malaria, however, and especially in quinin-resistant cases, it would appear that arsphenamin, neoarsphenamin and sulpharsphenamin are useful adjuvants to quinin, not so much by a direct plasmodocidal activity as by some mechanism tending to bring the parasites into the circulating blood, where they become more vulnerable to quinin. Furthermore, the arsenicals tend to correct the anemia of malaria and improve the metabolism and nutrition, especially of chronic cases; this is true, however, of the inorganic arsenical compounds as well, which have long enjoyed a place in the adjuvant treatment of the disease.

How arsphenamin and its substitutes aid in the specific treatment of malaria is not known. According to some observers the organic arsenicals increase the ameboid movements of the parasites and thereby bring them out into the circulating blood. In my opinion, the effects are rather due to the vasodilating properties of arsphenamin and its substitutes with the result that the parasites in corpuscles in the capillaries are dislodged. However that may be, there is no doubt that the compounds may produce Herxheimer-

like reactions in malaria and thereby aid in both diagnosis and treatment of chronic, latent infections. In addition, it is probable that the arsenic of these compounds interferes with the processes of oxidation and reduction, that is, the internal respiration of the parasites as originally claimed by Binz for explaining the toxicological effects of arsenic in general for protoplasm. Arsphenamin and neoarsphenamin, however, may combine with the malarial plasmodia through the affinity of the arsenic for sulphur compounds in the protoplasm of the parasites and bring about their destruction in a manner analogous to the mechanism involved in trypanocidal activity. However, if this is true, it would appear that the plasmodia contain much less sulphhydril compounds than trypanocomes and spirochetes, because the parasitropic effects are so much less for the former.

Quinin Prophylaxis of the Malarial Fevers.—In the prophylaxis of the malarial fevers most has been accomplished by antimosquito protection and the destruction of the pests, combined with the administration of quinin. There is no doubt of the efficacy of quinin prophylaxis, although in the malarial districts it is difficult and expensive to apply on a large scale and the prolonged ingestion of the drug tends to produce nervous irritability and tremors in some individuals. The advantages of quinin prophylaxis, however, are in excess of the disadvantages, and the practice has been strongly and universally recommended. Various methods or plans have been proposed, among which may be mentioned the following:

(*a*) Koch's method, which consists in taking 15 grains on two consecutive days every eight or ten days for three months.

(*b*) Celli's method, which consists in taking about 6 grains daily.

(*c*) Plehn's method, which consists in taking 8 grains every fourth day.

(*d*) Castellani and Chambers give 5 grains daily with a double dose or 10 grains every Sunday. For children 1 grain of quinin may be given for every three years of age, or euquinin in daily dose of 2 to 5 grains in a little sweetened milk, as advised by Celli, may be administered. In very malarious zones, such as certain tropical countries and the Balkans, they recommend larger doses than those advised above.

Since 0.1 to 0.2 gm. (2 to 3 grains) is usually sufficient for temporarily cleansing the blood of asexual forms of plasmodia, it would appear to me that the method of Castellani and Chambers is to be recommended, except that the dose may be reduced to 2 or 3 grains daily when the district is not very malarious and when the drug is to be taken over months and years of time.

Celli has estimated that in certain districts of southern Europe about 33 per cent. of the population developed malaria when no prophylactic measures of any kind were taken. Quinin prophylaxis alone reduced the incidence to 20 per cent.; antimosquito protection alone reduced it to 2.5 per cent., while quinin prophylaxis and antimosquito protection reduced it to 1.75 per cent.

Advantages and Disadvantages of Various Methods for the Administration of Cinchona Alkaloids in the Treatment of Malaria. *Method of Ingestion.*—Owing to rapid absorption of quinin from the stomach and intestines (especially the former) when these organs are in good condition, the oral administration of quinin has been long the favorite method of treatment and is undoubtedly of most value for the routine treatment of the malarial fevers except in very severe or malignant cases when intravenous injections may be required to bring the disease under control. But oral administration may be contra-indicated in some cases and possesses certain disadvantages. The bitter taste of quinin may be objectionable, but this can be usually overcome by administering the more soluble salts in chocolate covered tablets,

capsules or cachetes and for children the less bitter euquinin or quinin tannate may be given in capsules or cachetes or suspended in chocolate syrup or some other sweetened vehicle.

In cases of coma and vomiting the method of ingestion may not be advisable or possible, in which case intramuscular or intravenous injections may be required.

Furthermore, in vomiting the contents of the stomach may be rendered alkaline by the bile, in which case the absorption of quinin is hindered or fails to occur; under such circumstances, it may be first necessary to cleanse the stomach, and my colleague Dr. Yardumian, who has had an exceptionally rich experience in the treatment of malaria in Armenia and Turkey, states that hypoacidity or anacidity of the stomach and especially bilious vomiting with neutralization of the gastric juice, constitute very important reasons why quinin by ingestion may fail to cure malaria, although he believes that the method of ingestion is in the one of choice for the administration of the drug when these conditions receive appropriate attention and treatment and when the patient may be relied upon to actually swallow the drug or when it is possible to administer it under the supervision of a nurse or physician to prevent deception.

A large number of quinin preparations have been advocated for the treatment of malaria by ingestion. The sulphat is the cheapest, but probably the least desirable on account of being so lightly soluble (1:725) except in an acid medium. For adults whose stomachs are in good condition, the bisulphate or dihydrochlorid may be given in freshly prepared capsules. It is imperative, however, that old and hardened capsules, pills and tablets be avoided because of the possibility of these not dissolving and being absorbed. If the acidity of the gastric juice is reduced, it is advisable to use solutions. If the sulphat is employed, it is necessary to use about one minim of either sulphuric or hydrochloric acid for each grain for purposes of rendering it soluble, with the addition of some vehicle to aid in disguising the bitter taste, the following formula being satisfactory;

Quinin sulphatis	℥IV (15 gm.)
Acidi sulphurici dil.	℥V (20 c.c.)
Syr. aurantii cort.	℥I (30 c.c.)
Aq. Distil.	q.s.ad ℥iii (100 c.c.)
M. Dose 1 teaspoonful or 4 c.c.	

The bisulphat is much better, being acid and more soluble (1:9); likewise the dihydrochlorid, which is soluble 1:0.6.

Method of Intramuscular Injection.—This route of administration is always advisable when the patient cannot or will not swallow and in cases of severe vomiting. It is a mistake, however, to assume that absorption from the muscles may be more rapid than from the stomach. Fletcher, previously quoted, has recently stated that in Kuala Lumpus he gave 10 grains of the dihydrochlorid dissolved in 22 minims of salt solution to 22 patients by intramuscular (gluteal) injection. The shortest time that quinin appeared in the urine was twenty minutes and the average time was sixty minutes; the same amount of drug given by mouth was followed by the appearance of quinin in the urine in fifteen minutes, with an average of thirty-one minutes. He states that regardless of the alkaloid employed and its concentration, intramuscular injection is always followed by some degree of necrosis which in some cases may develop into an abscess. Furthermore, that large nerves may be involved, with great pain and at least temporary paralysis. He quotes Sir Patrick Manson as opposed to this route of administration except

for good reasons, and also states that Sir Ronald Ross had repeatedly declared himself against this route for the treatment of ordinary cases.

However, there are times and cases when intramuscular injections are indicated and required and I know that my friend S. Solis-Cohen has given quinin and urea hydrochlorid by intramuscular injection in the treatment of a large number of cases of malaria and pneumonia since 1893 with uniform success. His method consists in thoroughly sterilizing the water and syringe (all-glass preferred) and carefully preparing the skin by washing with soap and water and applying a coat of tincture of iodin. The drug (15 grains ordinarily) is dissolved in 2 to 5 c.c. of boiling water (20- to 50-per cent. concentration) and after cooling is injected deeply in the muscles, care being exercised to empty completely the syringe before withdrawal of the needle to avoid depositing any of the drug in the subcutaneous tissues. The iodin is then removed with alcohol and the puncture sealed with flexible collodion.

Some pain usually follows the injection, but I have prevented this in many instances by incorporating into the above solution 0.5 c.c of a sterile 1-per cent. solution of novacain or butyn. The following solution is also said to be less painful: 10 grams of quinin hydrochlorid and 5 grams of ethylurethane dissolved in 20 c.c. of distilled water, followed by sterilization. Each cubic centimeter contains approximately 0.5 gm. of quinin and the dose is ordinarily 2 c.c. or about 1 gm. (15 grains). It is always advisable, however, for physicians practicing in malarial districts to keep ampules of a sterile solution of quinin on hand and these are made available in splendidly prepared solutions by various commercial laboratories. Recently Macphail[43a] has again emphasized the value of intramuscular injections of 15 to 22½ grains of quinin dihydrochlorid in the treatment of serious cases of malaria and it would appear that this route of administration has the approval of many experts practicing in malarial districts.

Method of Intravenous Injection.—The intravenous injection of the cinchona alkaloids is accompanied by considerable danger and is certainly not to be regarded as a method of choice, but rather one of necessity to meet urgent situations. The sudden introduction of these compounds and especially concentrated solutions by syringe injection, which are so frequently described in text-books, may produce a great reduction of blood-pressure by cardiac depression and vasodilatation through peripheral action and it is particularly important to avoid this route of administration if the patient presents signs and symptoms of cerebral malaria, as the vasodilating effects of the meningeal vessels and depression of the respiratory center may be fatal. Likewise, when there are evidences of cardiac weakness or distress, great care is required and not infrequently in cases of pernicious malaria in which one is most tempted to use this route of administration, the blood-pressure is generally low, anyhow, on account of the infection. Furthermore, intravenous injections tend to produce distressing tremors and respiratory distress, and accidental perivascular infiltrations may produce local necrosis and sloughing.

However, against these dangers is the unquestionable rapidity with which the drug is brought in contact with the parasites in the blood, and Cantile and Moubarak[44] and others believe the method particularly indicated for the hospital treatment of malaria, since the maximum in results are obtained in the minimum of time. According to Maxcy,[45] its proper field of usefulness seems to be upon urgent clinical indications of two sorts: first, in cases in which prompt absorption by the gastro-intestinal tract, following mouth administration, is not to be expected, because of violent gastro-intestinal disturbance or other cause, or in which it is impossible to give the drug by

mouth on account of delirium, coma, etc; and second, in cases which are gravely ill when first seen by the physician and in whom it is deemed imperative to secure immediate cinchonization. It does not seem necessary or desirable to use the intravenous route of administration in the simple acute or chronic infections ordinarily encountered, whether tertian or aestivo-autumnal, because there is no clear evidence at present that in ordinary malarial infections the method is more effective than mouth administration in curing an acute attack, in ridding the blood of sexual forms, or in preventing relapse.

Both Maxcy and more recently Brahmachari[46] have emphasized the necessity of giving dilute solutions and injecting them slowly. The latter has studied the influence of injections of concentrated and diluted solutions on blood-pressure with interesting results. The injections were given in the apyrexial period, except in one case. He observed that the intravenous injection of quinin in concentrated solution (10 grains in 20 c.c.) was generally followed by a fall in blood-pressure and may cause a disappearance of the pulse for a few seconds. Intravenous injection of quinin in dilute solution (10 grains in 200 c.c.) might be also followed by a fall in blood-pressure, but this fall was neither as sudden nor as great as when the solution was concentrated; in many cases there was no reduction at all. The more slowly the injection was given the less likely was there to be a fall of blood-pressure. The diminished blood-pressure may persist for twelve hours or more after the injection. The author states that the intravenous injection of quinin should always be given in very dilute form (1:300) at the rate of 10 c.c. every minute and that it should never be undertaken lightly.

The dihydrochlorid is the salt generally employed. The dose is ordinarily 1 gram (15 grains) and this should be dissolved in at least 100 c.c. and preferably 200 c.c. of warm sterile, physiological saline solution and injected slowly by gravity in exactly the same manner as the intravenous injection of arsphenamin. It is well to watch the pulse and respiration very carefully and better still, to have the blood-pressure taken at intervals during the injection. It is also a good practice to have in readiness a syringe containing 1 c.c. of 1:1000 adrenalin chlorid: and in case of circulatory collapse, 0.1 or 0.2 c.c. of the solution may be injected into the quinin solution through the rubber tubing for intravenous administration or 0.5 to 1 c.c. may be injected intramuscularly. If the patient is hemorrhagic, that is, purpuric or passing blood or hemoglobin through the kidneys, it is well to dissolve the quinin in 100 c.c. of 0.5-per cent. solution of calcium chlorid in saline solution and inject the two drugs together, as will be discussed shortly with more detail in the treatment of blackwater fever.

While, however, the intravenous administration of dilute solutions by syringe injection appears to be the method of choice, it is well known that in practice it cannot always be applied and especially in the country districts. Furthermore, concentrated solutions have been injected too frequently and successfully to condemn the method. Bass, for example, has described a method consisting of dropping two 5 grain tablets of quinin dihydrochlorid in the barrel of a 20 c.c. syringe carrying a few cubic centimeters of sterile saline solution and, after dissolving, filling to the 20 c.c. mark and injecting. Castellani and Chambers described the injection of 15 grains of the same drug dissolved in 5 to 10 c.c. of saline solution. But if these methods are employed, the injection should be given slowly by means of a small needle like No. 22, in order to have thorough dilution with the blood.

Method of Rectal Injection.—Quinin is ordinarily so irritating to the rectum that this route of administration is of very little value. When the drug cannot be given by stomach, intramusclar injections are to be preferred.

If rectal administration is attempted, 20 grains of the dihydrochlorid may be dissolved in 3 ounces of starch solution containing a few drops of tincture of opium and injected as high as possible. Suppositories of quinin or quinoform may be employed in children.

General and Specific Treatment of Acute Malarial Fevers.—A very large number of plans or methods for the treatment of malaria with quinin have been proposed, different authors placing varying importance upon many factors, as the time when the drug should be given in relation to the paroxysms, the particular drug to use, the dosage, route of administration, etc. All of these are worthy of consideration since they are influenced by the kind of infection, its severity and duration, so that no hard and fast rules are permissible.

1. It is always advisable to examine the blood for the kind or kinds of plasmodia present and the proportion of infected corpuscles; for example, when 5 per cent. of cells are carrying parasites, it is apparent that a severe infection exists requiring intensive medication.

2. Calomel should be administered followed by a saline. In some unaccountable manner this drug appears to favor the absorption and activity of quinin. The heart should be examined and if weak or irregular with a weak pulse and low blood-pressure, due care must be exercised in the dosage and route of administration of quinin. Stimulants like 1 to 3 grains of caffein citrate by ingestion or subcutaneous injection may be indicated. In pregnancy, due attention must be given to the possible effects of quinin on the uterus and smaller doses than usual given in order to minimize the danger of exciting contractions. Sodium bromid in dose of 5 grains once or twice a day may be advisable for excitability or nervousness due to quinin or the infection.

3. For benign tertian and quartan fevers of adults, 10 grains of quinin bisulphate may be given three times a day or 15 grains twice a day until the temperature has been normal for a week or ten days. In severe tertian and quartan fevers and in all cases of pernicious malaria (aestivo-autumnal fever), it may be necessary to give 15 to 20 grains three times a day or to give one intramuscular injection of 15 grains of the dihydrochlorid or quinin and urea hydrochlorid and three 10-grain doses by ingestion per day until the infection is under control. In pernicious malaria daily intravenous injections of 10 to 15 grains of the dihydrochlorid may be required for the first three to five days, these being omitted and intramuscular injections substituted until the infection is brought under control; at the same time the bisulphat may be given orally in dose of 10 grains three times a day.

4. In typical malaria when the patient is first seen in an acute attack, the first dose of quinin may be given during the crisis when the fever is declining in order to attack the merozoites free in the blood or shortly after they have entered corpuscles.

5. Quinin may be given as described until the infection is under control; by this I mean the absence of fever and chills for at least seven to ten days along with negative blood smears.

6. From this time on 10 grains may be given twice daily for the first month, 10 grains once daily for the second month and 5 grains once daily for the third month; or the method advised by Rivas may be employed: 10 to 15 grains twice a day for three days in succession followed by a four day interval without quinin, these periods alternating for two or three months, followed by 10 grains daily for the balance of the season.

7. Certain symptoms may require special measures. *Headache*, for example, may require cold applications to the head; the coal tar products

like phenacetin and acetanilid should be avoided if possible. *Hyperpyrexia* is relieved by cold sponging. After the sweat it is well to change the clothes and give a sponge bath with alcohol or tepid water. *Vomiting* may be relieved by sips of iced water, limeade, etc., but if it continues the stomach should be washed out. It must not be overlooked that in the presence of vomiting, quinin may not be absorbed and that intramuscular injections may be required. During the *chills*, hot water bottles and blankets may be required. *Coughing*, *hiccough* and *diarrhea* may require appropriate treatment. In severe malaria, as in cases with cerebral symptoms, algidity, severe choleraic diarrhea and vomiting, scarlatiniform rashes, etc., indicating a high degree of toxemia, it is advisable to give intravenous injections of 500 to 800 c.c. of physiological saline solution; if these cannot be administered, it is advisable to inject the saline under the skin. These injections frequently yield splendid results and especially if free sweating occurs; they may be repeated once or twice daily. In hemorrhagic cases with purpura or hemoglobinemia and hemoglobinuria, it is well to add 0.5 to 1 gram (8 to 15 grains) of calcium chlorid to the saline for injection intravenously or subcutaneously and 0.5 to 1 c.c. of 1:1000 adrenalin may be injected subcutaneously if the heart and pulse are weak with low blood-pressure.

8. During the acute attacks the diet should be light and of fluids; in the asymptomatic periods and convalescence ordinary food may be given.

9. During convalescence it is necessary to see that the patient's bowels are evacuated once daily and a simple "tonic" mixture of iron and strychin may be useful.

General and Specific Treatment of Chronic and Relapsing Malarial Fevers.—It is frequently a very difficult matter to determine when malaria is completely cured in the sense of total extirpation of all plasmodia, and, indeed, if the disease has been present for several months before treatment has been begun, it is impossible to determine when complete cure has been obtained, since negative blood smears may be observed in cases in which parasites are lodged in the spleen and other organs. When the disease has been present for years these difficulties are greatly increased and complete cure frequently impossible, although relapses may be successfully combated by quinin therapy.

1. It is important to correct constipation and if chronic gastro-intestinal disturbances are present, possibly associated with hypoacidity of the gastric juice, it is advisable to administer the acid salts of quinin like the bisulphat or hydrochlorid, and their solution and absorption may be aided by giving 10 minims of dilute hydrochloric acid in water with each dose.

2. Relapses may be treated with quinin as described above for the specific treatment of acute malaria, but since these cases are particularly apt to develop an intolerance for the drug, it is well to treat rather intensively for a week at a time alternating with periods of a week without quinin. In other words, it would appear that the correct principle is to "hit hard" for a short period rather than lightly over a prolonged period. If intolerance is present, it is sometimes possible to administer one of the salts of cinchonin. For these cases with extreme quinin hypersensitiveness, it may be necessary to attempt desensitization.

In addition, it may be helpful to give 5 grains of methylene-blue in capsules three times as day; picric acid in dose of 2 grains two or three times a day by ingestion has also been recommended.

I am convinced that the intravenous injection of 0.4 gm. of arsphenamin or 0.6 gm. of neoarsphenamin is frequently helpful and these injections, especially of neoarsphenamin, may be repeated at weekly intervals until

the infection is again under control, as indicated by freedom from signs and symptoms with negative blood smears for at least a period of ten days. Furthermore, injections of neoarsphenamin are quite helpful for aiding in the treatment of malarial anemia and cachexia, as discussed with more detail in Chapter XXXIV.

Chemisse[47] believes that the internal administration of 5 to 10 drops of tincture of iodin, well diluted with cinnamon water, twice daily, should be given with the quinin; possibly the iodids may be substituted for the iodin.

3. After a relapse has subsided, it is well for the patient to take quinin periodically for months or years of time. But in order to reduce the chances of gradually evolving a quinin resistant strain, it would appear advisable to adhere to some plan whereby 5 to 10 grains are taken once a day for five to seven days every month rather than smaller doses more or less continuously. Of course, this is greatly influenced by the degree of infection and the tendency for relapses, but the principle of "hitting hard" for a short time with relatively large doses appears to be correct, both from the standpoint of controlling the infection and of avoiding the muscular tremors and other signs of chronic cinchonism.

As previously stated, it is impossible to determine when a complete cure has been effected, but, according to Thaller,[48] Habetin[49] and others, the intramuscular injection of sterilized milk in dose of 5 to 10 c.c. or subcutaneous injection of nucleic acid or sodium nucleinate sometimes provokes latent infections and brings the parasites into the blood stream where they may be detected by smear examinations.

According to Silvestri,[50] Schimert[51] and others, the intramuscular injection of milk and other agents, including intravenous injections of the patient's own serum, frequently aids treatment with quinin in chronic resistant cases.

4. In *chronic malaria with hemoglobinuria*, large doses of quinin are required since there are usually *P. falciparum* infections. But it is well to precede the quinin by one or two intravenous injections of 5 c.c. of a sterile 5-per cent. solution of calcium chlorid in water or saline and these may be given once a day for several days to control the hemorrhage; the injections must be given *very slowly* and with great care. Or calcium lactate in dose of 5 to 10 grains may be given by mouth before and during quinin administration.

5. As previously stated, I believe that the so-called *quinin hemoglobinuria*, sometimes developed by cases of chronic malaria, is really due to malarial infection. Sometimes it is sufficient to give 10 grains of calcium lactate by mouth before each dose of quinin, but if this fails it is usually possible to control the bleeding by the intravenous injection of 5 to 10 c.c. of sterile 5 per cent. solution of calcium chlorid once a day for three days while quinin is being administered to check the infection.

6. *Blackwater fever* is a far more serious manifestation of chronic malaria, calling for more vigorous treatment. It is advisable first to check the bleeding and flush out the kidneys and for this purpose 100 to 200 c.c. of a sterile 0.1 per cent. solution of calcium chlorid in physiological saline should be injected subcutaneously or intravenously (*slowly*) once or twice a day. After the first injection or two, quinin may be given and preferably in the form of an intramuscular injection once daily of 10 grains of the dihydrochlorid or quinin and urea hydrochlorid. If there is no vomiting, calcium lactate may be given orally in dose of 5 grains two or three times daily, and Castellani and Chambers also recommend the oral administration of the following Sternberg-Hearsay mixture in dose of one ounce every two to four

hours during the first twenty-four hours and then every three hours until the urine clears:

Liq. hydrargyri perchloridi			f℥III(12 c.c.)
Sodii bicarbonatis			℥I (4 gm.)
Aq. distil.	q.s.	ad	f℥vi (200 c.c.)

However, vomiting may be present and prove so troublesome as to require small doses of morphin and atropin. As a general rule, it is advisable to administer calomel followed by a saline at the onset of the attack, but if diarrhea supervenes, bismuth may be required.

The heart must be carefully watched and stimulated if required by digitalis or caffein. When quinin is administered, it may be necessary to give intramuscular injections of 0.5 to 1 c.c. of 1:1000 adrenalin to correct vasodilatation and intramuscular or subcutaneous injections of pituitrin (1 c.c.) have also been recommended.

Headache and hyperpyrexia are best treated with cold applications and bathing, since it is advisable to avoid drugs as much as possible.

Anuria always threatens in severe cases and the kidneys must be relieved by promoting the activity of the skin by hot wet packs and by free purgation. The backache may be relieved by dry cupping or hot fomentations, but if these fail, small doses of morphin may be required.

The patient should be encouraged to take plenty of water or lemonade, but if vomiting is present, dehydration and thirst should be corrected by subcutaneous or intravenous injections of calcium-sodium-chlorid solution aided by rectal infusions of saline. The Murphy drip method is sometimes useful for this purpose. The diet should be of fluids and as nutritious as possible.

7. Whenever possible it is advisable for chronic malaria cases to seek a change of residence to colder climates and this is especially advisable for those who have had attacks of malarial or quinin hemoglobinuria or the severer blackwater fever. A return to the tropics is always fraught with much danger.

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CHAPTER XXXIII

CHEMOTHERAPY OF LEISHMANIASIS, AMEBIASIS, PIROPLASMOSIS, SCHISTOSOMIASIS AND OTHER PROTOZOAN AND METAZOAN DISEASES OF MAN AND THE LOWER ANIMALS

As stated in the preceding chapter, our present knowledge of the specific treatment of protozoan and metazoan diseases of man and the lower animals, with the exception of these caused by trypanosomes and spirochetes, is almost entirely confined to clinical experience with certain drugs, rather than the result of systematic chemotherapeutic investigation. In not a few diseases caused by protozoa, however, and especially the various forms of leishmaniasis like kala-azar and oriental sore, the antimonyl salts and particularly tartar emetic act almost as specifics; and likewise in some of the metazoan infestations, as in the different types of schistosomiasis, these compounds possess a high degree of curative activity, so that they have as much right to be classed with the group of specifics and in the field of chemotherapy as the cinchona alkaloids in the treatment of the malarial fevers.

In this chapter, I wish to consider the remainder of those diseases caused by protozoa and metazoa in which a more or less specific therapy may be said to exist; I do not doubt that the curative activity of tartar emetic, emetin hydrochlorid and other compounds to be considered could be improved upon by orderly and systematized chemotherapeutic research and especially for those diseases transmissible to the lower animals which always offers

thereby a way and stimulus for experimental investigations. But before taking up the subject of treatment, it may be well to consider some of the more important pharmacological and toxicological properties of the antimonyls and of ipecac and its alkaloids.

Chemistry, Pharmacology and Toxicology of Antimony Compounds in Relation to the Chemotherapy of Protozoan and Metazoan Diseases.—While the toxicity, trypanocidal and spirocheticidal activity of antimony and potassium tartrate (tartar emetic), as well as some new compounds like sodium antimony thioglycollate and the triamid of antimony thioglycollic acid (antimony thioglycollamide) prepared by Abel, have been already discussed in earlier chapters, yet it may be of interest to discuss here the absorption, elimination and certain other pharmacological actions of antimony, as well as the toxicological effects for human beings, inasmuch as tartar emetic is used so extensively for the treatment of protozoan and metazoan infestations.

Chemistry of Antimony Compounds in Relation to Toxicity and Therapeutic Activity.—Tartar emetic has been mostly employed and but comparatively little work has been done on new antimonyls, but the results have indicated that some of the new compounds may be superior and this is highly desirable since it is quite apparent that tartar emetic is not by any means the ideal or satisfactory remedy for kala-azar and other forms of leishmaniasis, largely because of its toxicity and the intolerance developed by many individuals requiring a long series of injections. In Chapter XIII, I have already referred to the work of Rowntree and Abel, and Voegtlin and Smith, who have prepared new pentavalent and trivalent organic compounds of antimony, some of which were apparently superior to tartar emetic in the sense of being less toxic for the host while possessing a high degree of parasiticidal activity. Mention may also be made of the work of Fargher and Gray[1] who have studied the effect of variation of the basic radicle in the emetics, the replacement of tartaric acid by other suitable acids and the question of the relative merits of the true organic compounds of antimony and the emetics. The general conclusion was drawn that a variation in the base in the antimonyl tartrates considerably reduces the toxicity per unit of antimony, the most favorable salts being those of quinin and p-phenetidin, but the replacement of tartaric acid by other acids yielded little of pharmacologic interest. As shown by Brunner[2] in 1912, trivalent organic compounds are more toxic than pentavalent compounds, being similar in this respect to the organic arsenical compounds, and it may be recalled that Kolle and his associates, as well as Voegtlin and Smith, found that trivalent antimonyls, while more toxic for the host were likewise more trypanocidal and more rapid in effects than the pentavalent compounds.

Brahmachari[3] has more recently reported interesting studies upon the comparative toxicity of the antimonyl tartrates and some new aromatic compounds. Ammonium antimonyl tartrate was found to be the least toxic of all the tartrates, the presence of nitrogen in the basic radicle diminishing the toxicity of some of these compounds. Because of the high antimony content of the ammonium salt, its relatively low toxicity for the lower animals and likewise for human beings, and since it was found to possess a marked degree of therapeutic activity in the treatment of kala-azar, Brahmachari considers it superior to both potassium and sodium antimonyl tartrate. But some of the new organic aromatic antimonyls and particularly urea stibamine, were found less toxic and equally useful in the treatment of kala-azar. In addition to this compound he has described the preparation and toxicity of (1) phenyl stibinic acid; (2) acetyl-p-amino-phenyl stibinic acid; (3) stibamine and (4) stibhectine, and these, as well as the investigations previously referred to, indicate the great possibilities of improving the treatment of the various forms of leishmaniasis as well as of granuloma inguinale and other infections, by organic antimonyls and particularly trivalent compounds.

Absorption, Elimination and Toxicity of Tartar Emetic.—The absorption, elimination and pharmacological action of tartar emetic is quite similar to arsenic. The former, however, is absorbed more slowly and the dissociated antimony ion is more irritating to the stomach, the nauseating and emetic effects being due to direct local irritation of the stomach by the metal with the production of hyperemia and swelling of the mucosa, rather than the result of central action as formerly believed. The acidity of the gastric juice may facilitate dissociation but this occurs almost as rapidly in the intestines, on the skin or in the subcutaneous tissues. As a general rule, vomiting removes the compound from the stomach before passage into the intestines can occur except when very large doses are given when the intestinal irritation resembles the changes produced in the stomach. The acute and chronic gastro-intestinal lesions of poisoning, therefore, bear a resemblance to

those produced by arsenic. Furthermore, tartar emetic cannot be injected subcutaneously or intramuscularly in adequate doses on account of its irritant effects and when administered intravenously, due care must be exercised against perivascular extravasations, as in the administration of arsphenamin and neoarsphenamin. The nausea and vomiting of tartar emetic are due to its local irritant effects upon the gastric mucosa; likewise the colic and diarrhea, which are due to irritation of the intestinal mucosa. The increase of saliva and perspiration are secondary to nausea although a part of the increased activity of the salivary and other glands may be due to stimulation of these by elimination of the compound. When tartar emetic is given intravenously the elimination is largely by way of the urine, bile and gastro-intestinal mucosa as is true of the elimination of arsenic; with sufficiently large doses, the effects of gastro-intestinal irritation like nausea, griping, diarrhea and vomiting, may be produced. In just what form tartar emetic is eliminated, I am unable to state. Christopherson[4] is apparently of the opinion that the drug circulates and is eliminated unchanged, but I doubt that this is the case and surmise that dissociation occurs with the possible production of loose combinations of the metal with the plasma proteins.

Curiously the toxicity of tartar emetic by ingestion is higher than by intravenous injection, but this is largely because a portion of the drug is eliminated by the kidneys after intravenous administration with consequently less elimination of the gastro-intestinal tract and less irritation than when the drug is administered by swallowing.

Tartar emetic injected intravenously or administered by ingestion may produce the same cardiovascular effects as arsenic, since antimony acts upon the musculature of the heart and capillaries in the same manner producing depression of the heart and dilatation of the capillaries with a fall in blood-pressure; for this reason due care must be exercised in the administration of this compound to individuals with cardiac disease.

Tartar emetic, however, does not hemolyze or agglutinate erythrocytes either *in vitro* or *in vivo* as may the arsenical compounds. In my experiments concentrations *in vitro* as high as 1:20 had no effect upon either washed or unwashed corpuscles and the intravenous injection of 0.020 gm. per kilogram apparently produced no intravascular agglutination.

Tartar emetic administered by ingestion and intravenous injection either in the form of single large toxic doses or in small, repeated doses produces changes in the kidneys and liver quite similar to those produced by arsphenamin, neoarsphenamin and pentavalent compounds of arsenic. The vascular changes, however, are not as extreme; for example, in my experiments with tartar emetic administered to rabbits, I did not observe the typical large red kidneys produced by some of the inorganic and pentavalent compounds of arsenic. But the same kind and degree of epithelial degenerations in the kidney and liver have been observed sometimes amounting to fatty degeneration. The lungs show marked hyperemia and edema, but not the masses of agglutinated corpuscles produced by the arsenicals, nor have I noted the same changes in the meninges and suprarenal glands produced by arsphenamin. Brahmachari states that after the administration of a toxic dose of an antimonyl tartrate, the pathological changes are most marked in the lungs, kidneys, liver, pituitary and suprarenal glands, consisting chiefly of hemorrhages into the substance of these organs and destruction of their cellular elements. Similar changes were produced by toxic doses of the new aromatic organic antimonyls referred to above.

Antimony influences nutrition in somewhat the same manner as arsenic, but it is not employed for "tonic" effects as are compounds of the latter. Levy[5] states that the non-protein nitrogen of the blood may be increased in some cases given intravenous injections and very small quantities given repeatedly by ingestion are said to increase the glycogen and fat of the liver without apparently altering the nitrogen of the urine.

The maximum tolerated dose of tartar emetic for rats, rabbits and guinea-pigs by the slow intravenous injection of 1-per cent. solution is about 0.015 gm. per kilogram of weight. For a man weighing 70 kilograms or about 145 pounds this corresponds to 1.05 grams. The dose ordinarily given human beings by intravenous injection at one time is 5 to 10 c.c. of a 1-per cent. solution or 0.05 to 0.1 gm., so that these amounts are well within the range of safety, being 10 to 20 times under the maximum tolerated dose, and may be safely repeated. Doubtless some human beings are more susceptible than the average since fatal poisoning has followed the ingestion of amounts as small as 0.1 gm. (2 grains), so that it is never wise to give more than 1 or 2 c.c. of a 1-per cent. solution intravenously for the first dose, although the average dose is 5 to 10 c.c.

Ordinarily doses of these sizes produce no ill effects except occasionally a slight chill followed by rheumatoid pains in the joints associated with stiffness, especially involving the shoulders and usually noticeable in the mornings. Sometimes a slight metallic taste, slight nausea, giddiness, tachycardia, cough and tightness of the chest may be complained of as intolerance develops. When coughing, vomiting, colic, diarrhea and great debility occur, it is never safe to increase the dose but advisable to interrupt treatment until elimination has occurred. To the best of my knowledge acute toxic reactions of the arsenical

"nitritoid crisis" type do not occur, doubtless because the vasculotoxic effects are much less than produced by arsenic; intravascular agglutination does not occur at all and individuals are much less likely to have natural or acquired hypersensitiveness to antimony. The blood-pressure may be greatly reduced and tachycardia prove distressing. Lasky and Coleman[6] have reported ten deaths attributed to potassium antimony tartrate employed in the treatment of bilharziasis.

Randall[7] states that sodium antimony thioglycollate, which he has used successfully in the treatment of granuloma inguinale as a substitute for tartar emetic, is less toxic than the latter and ordinarily produces no symptoms at all in the usual dose of 5 to 10 c.c. of 1-per cent. solution by intravenous injection. Antimony thioglycollamide in dose of 0.08 gm. dissolved in 20 c.c. of sterile water and injected intramuscularly or intravenously every second day until as many as 15 to 20 injections have been given, is now preferred to the former salt.

Dosage and Administration of Antimony Compounds.—For the treatment of protozoan and metazoan diseases, intravenous injections are usually given because the dose by stomach must be so small in order to avoid nausea and vomiting due to local gastric irritation.

For intravenous injection tartar emetic, sodium antimonyl tartrate, sodium antimony thioglycollate, the triamid of antimony thioglycollic acid or urea-antimonyl tartrate may be employed in the form of 1-per cent. solutions in sterile distilled water or saline solution. The solutions are not bactericidal and must be sterilized by heating to 80°–100°C. for at least one half hour and preferably on each of two or three days in succession. Autoclaving is not permissible because dissociation may occur with a dangerous increase in toxicity. Solutions may be sterilized by filtration and this is the method commonly employed; I have also observed that if solutions are prepared with 0.3-per cent. phenol in water or saline and placed in a refrigerator for a day or two that chemical sterilization occurs without either heating or filtration.

The dose of tartar emetic is ordinarily 4 to 10 c.c. (0.04 to 0.1 gm.); according to Manson the largest tolerated dose is about 0.130 gm. (2 grains) equivalent to 13 c.c. of the 1-per cent. solution. Rogers advises about 2 c.c. or 0.02 gm. for each 10 pounds of weight and Faber and Schussler state that they have given a child of 27½ pounds as much as 0.1 gm. at one time, but these doses appear to be too large for most individuals and in my opinion the first dose should not be more than 2 c.c. since the tolerance of the patient is unknown. Commercial laboratories have prepared 1- or 2-per cent. sterile solutions in ampules ready for administration. The injections may be given by means of a syringe, as described in Chapter LIX, care being taken against accidental injection of the perivascular tissues. Doses of 5 c.c. may be given daily or at the rate of two or three per week without ill effects; as much as 30 to 40 injections, totally almost 2 grams of tartar emetic, having been injected without apparent untoward results.

Subcutaneous and intramuscular injections of tartar emetic cannot be given on account of the necrosing effects; I have tried small doses like 0.05 gm. in almond oil by intramuscular injection but the pain and disability is usually greater than follows injections of mercury. Martindale has employed injections of antimony oxid in dose of 150th grain in equal parts of glycerin and water; solutions of tartar emetic in phenolized water in dose of 0.5–1 c.c. carrying ½–1 grain (0.03–0.06 gm.) have likewise been employed. Randall states that antimony thioglycollamid may be administered by intramuscular or intravenous (preferred) injection every second day for as many as 15 to 25 injections, in dose of 0.08 gm. (about 1 grain) dissolved in 20 c.c. of sterile water.

The dose of tartar emetic by mouth is ordinarily about 0.005 to 0.008 gm. or 1/12th to 1/10th grain; larger doses are apt to produce nausea and emesis.

Oral administration is sometimes advised following a series of intravenous injections and Castellani and Chambers have employed the following formula:

Antimonii et potassii tartrati	gr. v (0.3 gm.)
Sodii bicarb.	gr. xxx (1.8 gm.)
Glycerini	
Aq. chloroformi	aa f℥i (30 c.c.)
Aq. distil.	q.s. ad f℥ iii (100 c.c.)

The dose is one to two teaspoonfuls in water three times a day.

Mechanism of Parasiticidal Activity of Antimony Compounds.—The mechanism of curative activity of tartar emetic in such protozoan diseases as the various forms of leishmaniasis and in such metazoan diseases as schistomiasis is as yet unknown. As discussed in Chapter XIII, Voegtlin and Smith are of the opinion that the pentavalent organic compounds are first reduced to a trivalent state before trypanocidal activity is produced. It is highly probable that all compounds become dissociated and that the antimony ions constitute the parasiticidal agents. Whether processes of oxidation occur with the production of new compounds analogous to the trivalent oxids of the organic arsenicals or whether union with the plasma proteins occurs with the formation of antimony-albuminates; cannot be stated. It is likely that the mechanism is similar to that shown by mercury in its spirocheticidal activity and that antimony ions directly attack the protoplasm of those parasites for which it has an affinity. Why antimony poisons the protoplasm of some protozoa and metazoa and not others cannot be stated, just as we are unable to explain the higher spirocheticidal effects of mercury for *Sp. pallida* of syphilis than for *Sp. pertenuis* of frambesia tropica (yaws).

Doubtless the readiness with which antimony compounds dissociate into antimony ions influences both parasitropic and organotropic effects. As in the case of mercurials I surmise that it is possible to classify the organic and inorganic compounds into the same classes of full complex, pseudo-complex and half complex compounds and that the most rapidly dissociated compounds will prove the most toxic for both the body cells and the parasites.

Since intravenous injections of tartar emetic may produce a slight leukocytosis, at least in rabbits, it is possible that the specific parasiticidal effects are enhanced or aided by certain non-specific agencies commonly designated as the non-specific protein reactions so commonly observed following the intravenous injection of vaccines and other protein substances as well as some of the metals in colloidal suspension. Whether or not tartar emetic stimulates antibody production, cannot be stated, since immunological investigations of this kind do not appear to have been made. As stated in Chapter XIII, I have observed a temporary resistance of about five days duration in rats to infection with *T. equiperdum* after the animals had been sterilized of a previous infection by tartar emetic. This resistance, however, may have been due to retained medicament and I very much doubt that the antimonyls stimulate immunological resistance and believe that the specific curative effects of these compounds are due to the action of ionic antimony aided possibly, by certain non-specific agencies in the way of leukocytosis and bone marrow stimulation; in short, that the mechanism is analogous to that described in Chapter XIV for mercury.

Chemistry, Pharmacology and Toxicology of Ipecac and Emetin, in Relation to the Chemotherapy of Protozoal and Metazoal Diseases.—The curative activity of ipecac and especially of emetin hydrochlorid in amebic dysentery has been well established and the latter especially has proven useful in the treatment of various other protozoan and metazoan diseases, particularly in

the various types of schistosomiases or bilharziases; indeed in the treatment of some of these diseases emetin is our main drug and for this reason is deserving of particular interest and study not only because of the practical results in treatment but likewise in relation to the possibility of improving the chemotherapy of these diseases.

Chemistry of Ipecac and Its Alkaloids, in Relation to the Chemotherapy of Protozoan and Metazoan Diseases.—While various preparations of ipecac root itself have undoubtedly proved amebacidal *in vitro* and curative for the amebic dysenteries, one of its alkaloids, emetin, has proved more efficacious and more useful. Harris, in 1819, claimed that ipecac more or less freed from its alkaloids ("de-emetinized") was equally effective and less disagreeable, but Vedder[8] and others have since proved that while they may be less disagreeable, they are apt to be therapeutically inert.

In other words the parasiticidal effects of ipecac are due almost entirely to its two most important alkaloids namely, emetin and cephaelin; psychotrin is much inferior. But both of these and particularly cephaelin, are capable of producing nausea and vomiting by local irritation of the stomach when swallowed and by central stimulation when injected and it would be a great triumph for chemistry to synthesize a new compound of equal parasiticidal activity but of lesser toxicity and especially for the gastro-intestinal tract and vomiting center. As a matter of fact, but little has been done in this promising and important field. It is true that various salts of emetin have been prepared but they are about equal in toxicity and for all practical purposes, the hydrochlorid is best for treatment. Furthermore, some emetin derivatives with higher alkyl esters have apparently proven more parasiticidal than emetin, at least for amebas *in vitro*, but so far no substitute has been offered for emetin.

Enough work has been done to show that emetin is the best constituent of ipecac for the treatment of amebic and other protozoal infections; nothing is apparently to be gained in the way of further efforts toward the isolation of other alkaloids or glucosides from the root, but it is possible that systematized chemotherapeutic investigations may result in the elaboration of new compounds of superior qualities and especially for encysted protozoa. Quinin, *chaparro amargosa* and other vegetable substances, as well as antimony and other metals, are also amebacidal, but the chemotherapy of the amebic infections as well as other protozoal and metazoal diseases is still in an unsatisfactory state. It is particularly desirable to discover compounds of greater parasiticidal activity and especially those of greater penetrability, in order to reach and destroy parasites and their ova enmeshed in the deeper tissues and especially those capable of encystment in the course of their life cycles.

Pharmacology and Toxicology of Ipecac and Emetin, in Relation to Methods of Administration.—Ipecac itself can be only administered by ingestion or rectal injection and since Rogers[9] in 1912, encouraged by Vedder's investigations, showed the superiority of emetin hydrochlorid by subcutaneous injection in the treatment of amebic dysentery, this alkaloid has almost supplanted preparations of the root. As shown by Eggleston and Hatcher[10] and others, cephaelin is more irritant and about twice as toxic and emetic as emetin; psychotrin is less toxic but likewise less parasiticidal, and ipecacuanhic acid, ipecacuanhin and various cephaelin esters have been found more or less inert, so that for all practical purposes, emetin is preferred and especially when subcutaneous injections are to be given.

Ipecac and emetin are quite irritant for the stomach and largely produce nausea and vomiting by local irritation, being analogous to tartar emetic in this respect, rather than by action upon the medullary center, since the dose of emetin by subcutaneous injection necessary to cause vomiting is larger than the emetic dose by ingestion. In order to overcome these nauseating and emetic effects, the drugs have been administered in various ways to get them through the stomach without irritation, as in the form of salol-coated pills or difficultly soluble preparations like emetin-fuller's earth adsorbate and emetin-bismuth iodid; also by rectal infusion, which, however, is of limited value since only a small part of the large intestine is reached unless the enema is given with special skill and methods.

For the treatment of amebic dysentery, however, these methods may be sufficient for bringing the drugs in contact with the parasites in the intestinal mucosa, but since absorption from the gastro-intestinal tract is slight, not enough emetin is likely to be absorbed for the treatment of urinary and pulmonary schistosomiasis or bilharziasis and other blood and tissue infestations with the parasitic trematodes. For the treatment of these diseases and other infestations of the blood and various internal organs by the parasitic trematodes, subcutaneous injections of emetin are very much to be preferred to oral administration.

Curiously, emetin injected subcutaneously is apparently much less irritating than when applied to the skin or mucous membranes. The nausea, vomiting and diarrhea are largely

due to irritation of the gastro-intestinal mucosa sometimes amounting to marked and severe inflammatory changes and hemorrhages; in the eye it may produce severe conjunctivitis, but the gums and buccal mucosa are more resistant permitting the local application of 0.5- to 1-per cent. solutions for the treatment of gingival amabiasis. Rectal and vaginal douches, however, may produce considerable irritation.

Strangely enough very little is known of the elimination of emetin and especially after subcutaneous injection. Lowin,[11] who has studied this subject with particular care, states that he could find no traces in either the urine or gastro-intestinal contents and Lyons[12] also failed to find it in the feces. But I feel that some of the drug must be eliminated in the urine since the kidneys may show acute hyperemia, hemorrhages and even epithelial degenerations in poisoning; furthermore, it would appear that elimination by this route affords an explanation for the curative effects of the drug in urinary bilharziasis due to the presence of *Schistosoma hæmatobium* and its eggs in the veins and tissues of the urinary bladder. Surely the gastro-enteritis is due in part to the elimination of the drug by the intestinal mucosa, for how else may we explain the curative effects of emetin in amebic dysentery and intestinal bilharziasis (caused by the worms and eggs of *Schistosoma mansoni*)? Furthermore, it would appear that a part of the drug may be eliminated in the bile, accounting for the parasiticidal effects in the intestinal canal and likewise for its curative action in hepatic bilharziasis or "Katayama disease," caused by the worms and ova of *Schistosoma japonicum*, as well as possibly, in liver infestations by *Fasciola hepatica* and *Fasciola gigantica*. On the other hand, however, it is well established that emetin injected subcutaneously or intravenously has no effect or almost no effect upon *Amœba coli* in the intestinal tract of man. This species is non-pathogenic, but experimental studies have shown that it is readily destroyed by ipecac and emetin *in vitro* and it is difficult to understand why these drugs have little or no influence upon it *in vivo*, even when the compounds are administered by mouth. Apparently emetin can only reach those amebas in the deeper tissues of the intestinal walls, but failure to remove those *Amœbae coli* and *Endamœbae histolytica* on the mucosa indicates that when ipecac and emetin are administered by stomach, absorption probably occurs in the upper portion of the small intestine leaving little or none in the intestinal contents reaching the colon unless large doses are given; also that if these drugs are eliminated at all in the ileum and colon, it must be only in traces too small under ordinary circumstances to appreciably influence the free living amebas and still less the encysted parasites.

Furthermore, it would appear that emetin injected subcutaneously is only very slowly eliminated; Udonda and Carulla, Savignac and Alivisatos and others have recorded instances of polyneuritis and urticaria due to a cumulative action of emetin in which it appears that the drug was retained for periods ranging from three months to more than a year. I shall refer shortly to this subject again as it bears an important influence upon the toxicity and administration of emetin.

The toxicity of emetin for the lower animals by subcutaneous and intravenous injection has been shown by Levy and Rountree[13] to vary greatly with different preparations of the alkaloid and when intravenous injections are given, the dilution of the drug, rate of injection and susceptibility of the animal employed, exert important influences. Vedder observed that 0.0025 gm. per kilogram was about the lethal dose for rabbits by intravenous injection; Levy and Rountree observed that from 0.004 to 0.018 gm. per kilogram was the lethal dose for dogs and cats, while Smith and I[14] found that by this route of administration, 0.016 gm. per kilogram was immediately lethal for rats and 0.010 to 0.0129 gm. per kilogram immediately lethal for rabbits. By subcutaneous injection Levy and Rowntree found that the lethal dose for dogs and cats varied from 0.003 to 0.029 gm. per kilogram according to the preparation used but as a general rule doses of 0.003 to 0.005 gm. per kilogram killed in from two to five days. If the average *maximum tolerated dose* by subcutaneous injection is taken as about 0.003 gm. per kilogram, corresponding to 0.210 gm. per 70 kilograms or 145 pounds, it is apparent that the usual adult dose of ½ grain (0.03 gm.) is well within the margin of safety, although repeated doses of this size have produced severe reactions and not a few deaths, especially in hyper-susceptible individuals.

Emetin injected subcutaneously or intravenously may cause a marked fall in blood-pressure mainly due, according to Levy and Rountree, to a direct toxic action on the heart with acute dilatation and death in diastole; vasomotor paralysis may also occur. Smaller doses produce cardiac irregularity due to fibrillation of the ventricles and the coagulation time of the blood is delayed. I have found no evidences of agglutination of erythrocytes in dilutions as low as 1:10 and, while washed corpuscles may undergo slight hemolysis at this concentration, whole blood is not hemolysed because of the protection afforded by the plasma. Chopra[15] has shown that in rabbits, marked histological changes may occur in the heart consisting of cloudy swelling, atrophy and fibrosis of the muscle cells. Emetin is also toxic for the capillary endothelium, producing marked degrees of petechial hemorrhage. After toxic doses, the kidneys may show a hemorrhagic type of nephritis with albuminuria; the liver usually shows acute hyperemia and cloudy swelling while the entire

gastro-intestinal tract from "cardia to anus" presents a severe hyperemia with hemorrhages and epithelial degenerations.

Emetin, therefore, is a drug requiring considerable care in administration and especially when repeated doses are required as is usually the case. Levy and Rountree have collected a large number of case reports of very severe reactions and several deaths; Lyons[16] has also recorded a number of toxic reactions and states that emetin should not be administered by mouth at all since even ½ grain doses may produce nausea, vomiting, griping and diarrhea.

Baermann and Heinemann[17] have noted distressing and even alarming symptoms following doses much larger than are usually employed. I believe that doses of ⅓ to ½ grain by subcutaneous injection are as large as demanded and that larger doses, especially when repeatedly given, are dangerous and uncalled for; intravenous injections are not usually indicated or required and should not exceed ¼ grain (0.015 gm.) for an adult of average weight. As stated by Lyons, the subcutaneous injection of ⅓ grain causes but little discomfort, but 1 grain doses may produce a severe inflammatory reaction lasting for one to two weeks. Since these reactions probably delay absorption, he advises small and repeated doses as being preferable to single large ones.

In human beings emetin may produce, therefore, a train of toxic symptoms consisting principally of such gastro-intestinal effects as nausea, vomiting, abdominal pain and diarrhea; such cardiovascular effects as arrythmia, fall in blood-pressure, petechial hemorrhages (including purpuric skin rashes), cerebral and pulmonary edema and such general effects as peripheral neuritis, extreme malaise and muscular debility. Polyneuritis is especially prominent. Some individuals are apparently hypersusceptible to the effects of ipecac and its alkaloids; skin tests have not been reported upon in these, but I surmise that they may be elicited just as in quinin allergy. At least it is always well to make the first dose of emetin very small (⅛ grain) in order to test the susceptibility of the patient before larger doses are given.

Mechanism of Parasiticidal Activity of Emetin.—Emetin and cephaelin are known to be very powerful amebacides and protoplasmic poisons in general *in vitro* for many protozoa and metazoa.

Experiments *in vitro* with cultures of ameba, with *Ameba histolytica* in dysenteric stools and with *Endameba gingivalis* in the pus of pyorrhea (Rigg's disease) have shown that dilutions of emetin 1:10,000 and even as high as 1:100,000 may destroy the parasites, with loss of motility, swelling and granular degeneration of the protoplasm. In other words, emetin apparently unites directly with the protoplasm of amebas and other parasites, including some of the bacteria and notably the anthrax bacillus, bringing about certain morphological changes and death. It has an affinity for certain parasites just as quinin is a general protoplasmic poison and especially for malarial parasites. When adult human subjects are given 0.05 gm. (about 1 grain) by subcutaneous injection, the concentration in the blood probably reaches at least 1:80,000 and when several doses of this size are administered at close intervals, the concentration is probably even greater so that we may assume that emetin destroys amebas and other parasites in the tissues by direct attack. At least, there is no evidence of the production of new compounds in the tissues or of any influence upon increasing antibody production. In other words, the action appears to be a direct one just as quinin probably destroys the malarial parasites by direct toxicity. Dale and Dobell,[18] however, do not agree with this hypothesis. In their experiments with *E. histolytica* infestations of the colon of young kittens, they observed *in vitro* that these parasites would sometimes withstand the action of emetin at 1:1000 and even 1:100 for an hour; in no case was any appreciable effect produced by a 1:10,000 solution. On the basis of these observations they have abandoned the idea that the curative activity of emetin is due to more or less direct action on the ameba, but suggest that emetin may produce some obscure alteration of the tissues of the host, rendering them perhaps more resistant to the cytolytic action of the amebae.

Amebas and other parasites capable of encystment may resist the effects of emetin; apparently the drug is unable to penetrate the parasites just as quinin is ineffectual for the sexual types of malaria and especially of *Plasmodium falciparum*. Probably future chemotherapeutic researches will disclose a means of combining emetin with a dye or some other substance capable in greater degree of piercing these encysted or resistant forms; at least, there is urgent need for studies of this kind to improve the emetin treatment of amebic and other infestments and especially for the treatment of their relapsing and chronic diseases.

Dosage and Administration of Emetin.—Emetin hydrochlorid is generally administered by *subcutaneous injection* in the treatment of amebic dysentery and other protozoan and metazoan diseases. A great difference in opinion has been expressed in regards to dosage, probably because different observers have worked with preparations of emetin varying greatly in toxicity, as so well shown by the investigation of Levy and Rountree referred to above. It would appear, however, that the average dose for an adult is ⅓ to ½ grain (0.02 to 0.03 gm.) by daily injection not exceeding a total of 15 grains in any one series of injections on account of the danger of cumulative effects and especially the production of polyneuritis. If 1 grain is given at one time, it is my practice to divide this dose into two injections in order to avoid the severe local reaction that may follow depositing 1 grain in the tissues. I have observed, however, that different makes of emetin hydrochlorid have varied in this regard and some may be given in dose of 1 grain without any more local reaction than follows an injection of quinin and urea hydrochlorid. Intramuscular injections are said to be more painful. Dosage, however, depends upon individual cases, but as a general rule it is inadvisable to give more than 10 to 15 consecutive daily injections.

Intravenous injections are sometimes employed. The dose should not be over ¼ grain (0.015 gm.) dissolved in at least 10 to 20 c.c. of sterile saline solution or water and very *slowly* injected. Concentrated solutions have been given, but diluted solutions are to be preferred, just as in the administration of quinin. It is well to have in readiness a 1:1000 solution of adrenalin and to inject 0.5 c.c. intramuscularly if cardiac depression and vasomotor paralysis develops.

Oral administration may be given, but the dose should not be over ⅙ to ½ grain (0.01 to 0.03 gm.). Keratin or salol coated tablets should be used, in order to pass the drug through the stomach into the intestines and thereby reduce the chances of producing local irritation, nausea and vomiting.

Colonic irrigations have been employed as 0.1 gm. of emetin dissolved in 1000 c.c. of water or saline (1:10,000 solution). The practical value of colonic irrigations with solutions of emetin or ipecac has been questioned, however, since the vegetative amebas are easily destroyed by irrigations with quinin, salicylic acid, silver nitrate, etc., without the danger of producing the direct irritation of the colon by emetin or ipecac, constituting the so-called "intestinal intolerance" for these compounds.

Chemotherapy of Leishmaniasis.—Since the organic arsenicals and especially arsphenamin and neoarsphenamin have yielded such excellent and encouraging results in natural and experimental spirochetal and trypanosonial infections of man and the lower animals, it was expected that these compounds would prove effective in diseases caused by other flagellates. But such has not proven to be true. For example, belonging to the family of trypanosomes is the genus *Leishmania*, parasites bearing a strong resemblance to the trypanosomes, except that they have no undulating membrane at any stage of development and for which arsphenamin and neoarsphenamin are ineffective while antimony compounds are so highly destructive as to constitute a specific form of therapy. "Leishmaniasis," therefore, is a general name including several diseases caused by different species of the genus *Leishmania*, just as the term "spirochetosis" includes several diseases due to different species of spirochetes, and as the term "trypanosomiasis" includes a number of diseases caused by different species of trypanosomes.

The parasites live chiefly inside of endothelial cells, but may be found in leukocytes or within the cells of the internal organs, such as the liver, spleen, etc. In the intracellular stage the parasites appear as non-flagellated, small,

oval, cytoplasmic bodies provided with a trophonucleus and a kinetonucleus. Several species have been cultivated on blood agar *in vitro* in which they regain the flagella.

Leishmania have been found in man, horses, rabbits, and other mammals inside of the endothelial cells of the capillaries of the liver, spleen, bone marrow, lymphatic glands, intestinal mucosa, in the blood of the femoral, portal and hepatic veins and sometimes in the peripheral blood, especially just before death, either free or within leukocytes. Infection is known to take place by direct contact and may be transmitted through an intermediate host, as bed-bugs, mosquitoes and other insects.

Diseases Produced by Leishmania.—Just as many pathogenic species of trypanosomes, spirochetes and other flagellates are known to occur in man and the lower animals, so there are likewise several pathogenic species of *Leishmania*. Our knowledge of these, however, is still incomplete insofar as differentiating among the various kinds is concerned, although their morphology and life history has been carefully and extensively studied. Among the species known to produce disease are the following:

(1) *L. donovani*, which produces internal leishmaniasis or *tropical* (*Indian*) *kala-azar*. *L. donovani* var. *archibaldi* is a subvariety of the same parasite.

(2) *L. infantum*, which produces kala-azar of children designated as *Mediterranean kala-azar*. *Canine kala-azar* is probably caused by the same parasite.

(3) *L. tropica* (Wright), which causes a cutaneous infection variously known as "*oriental sore*," "*Bagdad sore*," "*Delhi sore*," "*Aleppo boil*," etc. This species probably includes a subvariety called *L. americana* or *brasiliensis*, producing "espundia," a mucocutaneous infection in South America, and a similar disease in India and neighboring countries, called "Indian oropharyngeal leishmaniasis." Probably *L. nilotica*, producing a non-ulcerating keloid-like lesion designated as "Leishman's nodules" is another subvariety of this parasite.

While the chief endemic centers of Leishmania diseases according to Laveran[19] are the Ganges basin and Madras in India, China between the Yang-tse river, Peking and the Yellow Sea, and the Mediterranean littoral, yet *L. tropica* infections are quite common in South America, especially involving most of the continent east of the Andes between Panama and latitude 35°S. Cases of both kala-azar and oriental sore have been reported in the United States by Wright[20] who first described and established the organism in oriental sore, MacEwen,[21] Spencer,[22] Smith,[23] Fox,[24] Talbot and Lyon,[25] Faber and Schussler,[26] and Lambert.[27] Indeed, cases of leishmaniasis and particularly of oriental sore may be much more frequent in this country than surmized and especially since the prolonged incubation period may readily enough permit individuals from the endemic areas arriving in this country without lesions to subsequently dvelop the disease. Furthermore, oriental sores may readily escape detection and from the standpoint of laboratory diagnosis, biopsies with the examination of sections of tissue for the organisms are to be preferred to smears and cultures.

Whether the type of ulcerating granuloma of the genital organs and groins of both sexes and especially prevalent among Negroes designated as *granuloma inguinale*, is another form of cutaneous leishmaniasis, is not definitely settled. This disease has been known for many years, but has been confused with the venereal ulcers caused by *Sp. pallida*, *B. ducrey* and unidentified types. In 1904, Donovan[28] described certain inclusions in the mononuclear cells found in the lesions which have since become widely known as the "Donovan bodies" and generally regarded as the cause of the infection. Donovan regarded these organisms as protozoa; Flu[29] believes that they are chlamydozoa and might belong to the capsulated bacilli, while Carter[30] regards them as a gregarine type of crithidia or herpetomonads. Aragao and Vianna[31] also regard them as bacteria, but belonging to a new type, designated as "kalymmato granulomatis;" Walker,[32] Randall, Small and Belk[33] and Small and Julianelli[34] regard the organism as a bacterium of the *Bacillus mucosus* group. Certainly the spirochetes described by Wise,[35] Maitland[36] and MacLennon[37] are not to be regarded as the etiological agent and while the exact nature of the inclusion bodies is unknown, I believe that they are probably protozoa belonging to the genus *Leishmania;* this opinion, however, is largely based upon the remarkable curative properties of tartar emetic for the infection which exerts a similar effect in kala-azar, oriental sore and other forms of leishmaniasis. At least I believe it may be stated that, if *granuloma inguinale* is a bacterial infection, treatment with tartar emetic constitutes the most successful form of chemotherapy in a bacterial disease known to-day, and since similar bodies have been

found in the various cutaneous and mucocutaneous lesions of other *Leishmania* infections, I believe that it is highly probable that *granuloma inguinale* is of similar origin. In this connection I may state that Johns has recently failed to cultivate the Donovan bodies and is inclined to believe that they are not of bacterial nature.

Why there should be such a diversity of lesions and diseases due to such closely related varieties of *Leishmania* cannot be stated, just as we are unable to explain why frambesia tropica or yaws is almost entirely a cutaneous or mucocutaneous disease caused by a spirochete so closely similar to *Sp. pallida* that only cross-immunity or protection tests serve for differentiation. Indeed, the number of varieties and subvarieties of *Leishmania* appears to be constantly increasing, but it is highly probable that future investigations will show that while a number of clinical lesions or diseases may be produced, the number or varieties of *Leishmania* is not as great as surmised; for example, it is stated that monkeys inoculated with *L. tropica* are protected partially or completely against *L. donovani*, which shows the very close relationship between the parasites of these two well-defined diseases. On the other hand, Brahmachari[38] has recently described a cutaneous form of kala-azar (*L. donovani*) occurring in persons incompletely cured, which does not resemble oriental sore in any respect, and it is stated that some cases of oriental sore and espundia may have systemic lesions and symptoms which are not like those of kala-azar. Faber and Schussler have also mentioned that at least one case has been known to have become infected at different times by *L. donovani* of kala-azar and *L. tropica* of oriental sore, indicating that the parasites were not closely related; but as stated above, monkey cross-inoculation tests have shown that cross-immunization may occur and it is likely that, in the case referred to, the immunity conferred by the first infection disappeared or that superinfection is possible in leishmaniasis, just as it appears to be in some cases of syphilis.

Pathology and Immunity of Leishmania in Relation to Chemotherapy.—All varieties of *Leishmania* exhibit the same propensity for growing inside of cells and especially the endothelial cells of the vascular and lymphatic organs. In tropical kala-azar the vascular and lymphatic spaces of the liver, spleen, bone marrow and lymphatic glands are principally involved although the pancreas, kidneys, suprarenals, testicles, lungs and other organs may be similarly affected. In infantile kala-azar the spleen and liver are especially involved, although the lesions may be so similar to those in tropical or Indian kala-azar that this special clinical type may constitute nothing more than an infantile or juvenile variety of the disease. The parasites are apparently deposited in the capillaries and lymphatics by trauma and direct contact or by the bites of infected insects; apparently the disease is not congenitally transmissible, although when the testicles are involved the possibility of infection of the female exists with either paternal or maternal infection of the ovum or fetus. The parasites may remain at the site of inoculation and produce nothing more than a local sore or lesion with involvement of the neighboring lymphatics, as in oriental sore and granuloma inguinale. Or they may be carried to the internal organs and especially the spleen and liver, where endothelial cells are invaded; as these rupture, the parasites are discharged into the blood and carried to other organs. Toxins are apparently produced responsible in part for the cloudy swelling, fatty degeneration and even necrosis of the parts infected, as in the intestinal and other mucous membranes and the skin, although these changes may be due in part to the cutting off of the blood supply by the swollen endothelial cells of the capillaries; as would be expected fibrosis is produced in the chronic lesions. I have briefly reviewed this subject of the pathological changes to

indicate that, in the treatment of the disease, a drug like tartar emetic gaining access to the blood and lymph must penetrate the endothelial cells to reach the parasites and that this action may be still further hindered by fibrous tissue encapsulations; in other words, that treatment must be prolonged and that rapid sterilization is not to be expected except in the more recent and acute infections.

As is true of other protozoan infections like trypanosomiasis, syphilis and malaria, immunological resistance is not highly developed and the natural cure of man infected with *L. donovani* and *L. infantum* at least, is quite rare. It would appear, however, that one attack of oriental sore confers an immunity against the disease and it is stated that the Bagdad Jews have inoculated their children in the legs, in order that disfiguring lesions on the face, resulting from infection acquired in the usual way, might be prevented.

Cases of oriental sore and granuloma inguinale may recover frequently without medicinal aid, but only very slowly and usually with considerable tissue destruction. Some immunological resistance, however, may be acquired in all varieties of leishmaniasis responsible for holding the infection in check, but as a general rule this is gradually exhausted or overcome with progression of the disease. I mention these factors to show that medicinal treatment is required for recovery and that antimony apparently kills the *Leishmania* by direct toxic effect rather than indirectly by stimulating the production of antibodies or other specific immunological agencies.

Specific and General Treatment of Internal Leishmaniasis; (Tropical or Indian Kala-azar and Mediterranean or Infantile Kala-azar).—Tartar emetic (antimony and potassium tartrate) acts as a specific in the treatment of this disease; other antimonyls like sodium antimonyl tartrate and various colloidal antimonyls have also yielded excellent results as would be expected since the dissociated antimony ion appears to be the curative agent. Naturally a large number of other agents have been tried and Noguchi[39] has recently described an extensive series of experiments *in vitro* and *in vivo* with numerous antimony, bismuth, arsenical and dye compounds. According to Jemma[40] who has published an excellent historical review of the use of antimonyls in the treatment of leishmaniasis, tartar emetic was first employed by Caronia and Di Cristina. Brahmachari[41] prefers urea-antimonyl tartrate by intravenous and intramuscular injection in the same or slightly larger dose than tartar emetic and states that, while the therapeutic results are just as good, the untoward or distressing after-effects are much less, although in his opinion the best antimonyl for the treatment of kala-azar has not yet been discovered.

As a general rule, the drugs are administered by intravenous injection in the form of sterile 1-per cent. solutions. The average dose for adults is 5 to 10 c.c.; children from one to five years may receive 1 to2 c.c. and from five to twelve years from 3 to 5 c.c. Smyly[42] has recently stated that in experimental infections in rabbits only intravenous injections are of value; intramuscular and intraperitoneal injections were found much less efficacious.

The injections are generally given daily for 5 to 10 doses; then every other day for about 10 more doses and finally once or twice a week until a cure is effected. Intramuscular injections may be substituted, although these are apt to prove quite painful. Oral administrations may supplant these injections if the drug is well borne and are especially useful as a form of "follow up" treatment after a series of intravenous injections. Indeed, after the disappearance of lesions it would appear advisable to give a short series of 3 or 4 intravenous injections at intervals of a week twice a year for two or three years to insure against relapses in case complete sterilization has not

been secured or is impossible because of the chronicity of the lesions or drug intolerance. Atoxyl, arsphenamin and other arsenicals, as well as quinin, methylene-blue and other compounds have been employed but without success.

The heart must be carefully watched when intravenous injections of the antimonyls are being given, intramuscular injections of 1:1000 adrenalin in dose of 0.5 to 1 c.c. being especially useful for counteracting the vasodilating effects and drop in blood-pressure. Hemorrhagic lesions may be combated by the oral administration of calcium lactate in dose of 10 grains three times a day or by intravenous injections of 5–10 c.c. of 10-per cent. solutions of calcium chlorid. Arsphenamin and its substitutes apparently do not exert any marked specific effects upon *Leishmania*, but may be of service for correcting the anema usually associated with both the tropical and infantile types of kala-azar.

Specific and Local Treatment of External Leishmaniasis (Oriental Sore, Espundia and Indian Oropharyngeal Leishmaniasis).—Tartar emetic and other antimonyls are likewise regarded as specifics for the treatment of this type of leishmaniasis. As a general rule, intravenous injections of a sterile 1-per cent. solution are given in dose of 5 to 10 c.c. every day for five to ten days followed by ten to twenty additional injections every other day and finally once a week until a total of fifteen to thirty injections have been given. Intramuscular injections may be substituted and especially in young children, but are apt to prove quite painful. Oral administration may be given as a "follow up" form of treatment.

Excision of the lesions has been advised when they are so located that a rather wide excision can be made to remove infected capillaries and lymphatics, without too much deformity or tissue loss. This would appear to be an excellent practice, being similar to the excision of the malignant pustule or local anthrax lesion. After excision it is advisable to apply a 1-per cent. solution of tartar emetic for the local destruction of the parasites, but the powder itself produces too much pain. Various other local applications have been advised as potassium permanganate, methylene-blue, silver nitrate and other silver preparations, etc. Even though excision is done, it would appear advisable to follow with intravenous injections of tartar emetic to destroy parasites escaping surgical removal and especially those in the neighboring lymphatics; recurrences have sometimes occurred at the margins of the wound following excision when parasites remained in the tissues.

Oriental sores tend to heal spontaneously, but the course of the infection is so prolonged that specific treatment is always advisable not only to shorten the duration of the disease but as a safeguard against autoinoculation and transmission to others. Various local applications like 2- to 15-per cent. tartar emetic in lanolin may aid, but this particular preparation tends to produce irritation with the production of pustules, and intravenous injections of tartar emetic or some other antimonyl appears to be the best treatment. Scott[43] reports that injections twice or three times a week starting with 0.06 gm. (6 c.c. of 1 per cent. solution) and increasing by 1 or 2 c.c. gives best results and that in early cases before ulcer formation, complete cures are to be obtained in ten to fourteen days. Castellani[44] has recently stated that good results have followed local applications of ordinary phosphorus oil (B.P.) together with the injection of 3 to 5 minims under the skin around the nodule or into the lesion itself. *X*-ray treatment, freezing the lesions with carbon dioxid, etc., have also yielded good results in the hands of Adams and others.

Antimony Fastness of Leishmania in Relation to Treatment.—As stated in Chapter XIII, it is well known that various pathogenic trypanosomes may

be rendered antimony-resistant and it would appear that the same possibility exists in relation to the treatment of kala-azar and cutaneous leishmaniasis. According to Nasso and Mallardi[45] the doses of tartar emetic have to be increased to 0.05 or 0.07 gm. with longer intervals between injections, when this condition is developed. When resistance to tartar emetic is combined with acquired intolerance, they advise an arrest of treatment for several weeks to be followed by a series of small doses every two or three days and state that no child needs to die from leishmaniasis, if the administrations of tartar emetic are carefully and skilfully given.

Criteria of Cure of Leishmaniasis.—In the kala-azars it is advised by Struthers,[46] Muir[47] and others that specific treatment be continued until there are (1) an increase in weight and general well-being with a healthy state; (2) a normal temperature for at least six to eight weeks; (3) a reduction in the size of the spleen and (4) an increase in the leukocytes and hemoglobin. Rogers[48] lays great stress upon the leukocyte-erythrocyte ratio and when this is 1:1,500 or less, active kala-azar is almost certainly present. When conditions permit, at least one splenic puncture may be advisable for the detection of parasites before treatment is stopped and it may be that Napier's[49] gel-test is of value as a criterion of cure when it is gradually reduced from a positive to a permanently negatively reacting state.

Specific Treatment of Granuloma Inguinale and Other Granulating Ulcers.—The use of tartar emetic in the treatment of these ulcerating granulomata is credited to Aragao and Vianna[50] and since then numerous reports by Randall, Small and Belk,[51] Low and Newham,[52] Cummings,[53] Hoffman,[54] Reed and Wolf,[55] Cuthbert,[56] Lynch,[57] Schochet,[58] Johns[59] and others indicate that intravenous injections of tartar emetic frequently, and indeed usually, brings about a rapid cure of granuloma inguinale showing the presence of Donovan's bodies in smears of the lesions or in sections of excised tissue. Furthermore, as shown by the reports of Lynch and of Pereira,[60] this drug has effected the cure of other granulomata and granulating ulcers as cachar sores and leg ulcers in which these bodies have not been found, indicating that the antimonyls may have a parasiticidal effect in a variety of infections of this class. Syphilitic ulcers, of course, are not benefited by antimony and rapidly heal under treatment with arsphenamin and its substitutes. Chancroids may be benefited to some extent by injections of tartar emetic associated with appropriate local measures, but best results with the antimonyls have been secured in granuloma inguinale showing Donovan's bodies in the mononuclear cells. Local treatment for this infection has generally failed, although complete excision of the ulcers has been occasionally successful.

Sterile 1-per cent. solutions of tartar emetic in water or saline have been generally employed by intravenous injections. The first dose may be 2 c.c. followed at daily intervals by 4 to 10 more injections in dose of 5 to 10 c.c. each according to the tolerance of the patient for the drug. In some cases this treatment is sufficient, but extensive and long standing cases will require a longer series of injections at the rate of 2 or 3 per week and finally one per week, until as many as 15 to 30 injections have been given. Even after healing is complete, it may be well to give an additional series of 3 or 4 injections at weekly intervals to insure a complete and permanent cure, if the patient bears well with the drug and if there are no signs of renal irritation. Dosage should be carefully arranged and controlled for each individual case, including a careful examination of the urine for albumin, casts and erythrocytes at least once a week. As previously stated Randall[61] has also employed intravenous injections of antimony thioglycollamide in dose of 0.08 gm. dissolved

in 20 c.c. of sterile distilled water. As many as 15 to 25 injections are given at two day intervals and he recommends that at least 12 injections be given after the first healing has taken place to insure permanent cure.

Chemotherapy of Amebic Dysentery and Other Amebic Infestments.—While a host of pathogenic endamebas have been described varying from one another morphologically in greater or lesser degree, probably only four need command our attention from the standpoint of chemotherapy, namely, (*a*) *Endamœba histolytica* and (*b*) *Endamœba tetragena*, producing the dysenteries, liver absesses and other complications; (*c*) *Paramœba hominis* (Craig) and (*d*) *Endamœba gingivalis* (Gros) sometimes associated with Rigg's disease (pyorrhæa gingivalis). *Amœba coli*, probably the best known of all, is now generally considered a saphrophyte and incapable of producing dysentery and associated lesions. In the United States many physicians, indeed, probably the majority, have paid scant attention to amebic dysentery, because they have regarded it as an extreme rarity. Nevertheless, the disease, with liver abscess as a frequent complication, occurs over the greater part of the United States and particularly along the Pacific sea board and in the southern states.

Pathology and Immunology of Amebic Dysentery in Relation to Chemotherapy.—Why *Amœba coli* contents itself with remaining on the surface of the intestinal mucosa while *Endamœba histolytica* and other pathogenic types burrow through the mucosa into the submucosa cannot be stated; but this mainly represents the difference between non-pathogenic and pathogenic organisms of this class. Practically nothing at all is known of the agencies by which *Endamœba histolytica* gains access to the deeper tissues with the production of inflammatory changes; I doubt if this is merely a matter of motility on the part of the parasites and surmize that these pathogenic species are capable of secreting a toxin producing cellular necrosis and that the organisms are thereby able to open up an avenue of infection for themselves without the necessity for minute traumatic breaks in the epithelial barrier of the mucosa. Furthermore, it would appear that these toxic substances, as well as the protoplasm of the parasites themselves, are capable of eliciting inflammatory changes, although the bacteria and especially *B. coli*, carried along with the amebas or following in their wake may contribute in large part to the suppurative changes and the production of the acute and chronic ulcers of the intestine so characteristic of the disease.

Sometimes the amebas gain access to the radicles of the portal vein and, being carried to the liver, produce hepatitis and abscesses; sometimes the branches of the mesenteric arteries are invaded with the production of thrombosis and abscesses have been known to occur in the spleen, salivary glands and even in the brain. Petzetakis[62] has recently described cases of amebic bronchitis in which this distribution by the general circulation is suggested, although in cases of this kind direct infestation by the endamebas of the mouth may be responsible; Hemburger[63] has recently described amebic infestments of the skin.

As a general rule, the parasites are to be found in the margins of the ulcers and abscesses rather deeply buried in the tissues and for this reason local applications by means of colonic irrigations may fail to reach them and completely sterilize the tissues; fortunately, however, these deeply placed organisms are reachable by emetin injected subcutaneously, until fibrosis begins to cut off capillaries and access of drugs in the blood to the parasites, which accounts in part for the chronicity of amebic dysentery.

From the standpoint of pathological tissue changes, therefore, amebic dysentery offers numerous obstacles to successful therapy. In the first place, local application, while helpful, may fail to reach and destroy the organisms deeply buried in the margins of the ulcers. In the second place, secondary bacterial infection of the ulcers with *B. coli* and other intestinal bacteria is always present and contributes in no small part to the chronicity of the lesions. In the third place, endamebas in necrotic or fibrotic tissue may escape destruction by emetin and thereby greatly prolong the infection. For these and less important reasons amebic dysentery is a dangerous infection and aways likely to pursue a chronic course, requiring prolonged treatment even after acute symptoms have subsided, since relapses are so likely to occur, unless energetic treatment is given in the early stages before extensive tissue destruction has taken place. But secondary bacterial infections, in my opinion, constitutes the most important reason why the emetin treatment of amebic dysentery has so frequently failed and why so many observers have reported poor results. As shown by Vedder and since amply confirmed, ipecac and emetin are probably not at all bactericidal *in vivo* and I doubt if the chemotherapy of amebic dysentery, and especially chronic dysentery, will ever be on a thoroughly satisfactory basis until we discover a way and means

for more successfully combating the secondary and associated bacterial infections. In pure amebic infestations, as in liver abscesses, and likewise in early dysentery before bacterial infection has become important, emetin has proved very efficacious and a notable achievement in the chemotherapy of this disease, thanks to the investigations of Vedder and Rogers.

Little or nothing is known of immunological changes in amebic infestations. Apparently, however, spontaneous and complete recovery from well developed amebic dysentery is quite uncommon. It would appear that our body cells are not capable of producing appreciable amounts of amebacidal antibodies and this is analogous to the lack of antibody-production in other protozoan infestations. Doubtless cases of minor infestation recover and escape from the disease altogether is of frequent occurrence, but these are due to fortuitous circumstances rather than to immunological resistance. Sellards and Leiva[64] have recently stated that clinical evidence is very convincing that normal individuals exhibit considerable natural resistance to *Endamœba histolytica;* certainly the lower animals are highly resistant, young kittens being the only animals successfully infested for experimental purposes. But just how much this is due to natural antibodies or other immunological agencies and how much to the acidity of the gastric juice or local factors in the colon capable of destroying the free or encysted endamebas, is difficult to state and remains to be determined. But at any rate, I believe clinical experience has shown that recovery from amebic dysentery is usually a long process when unaided by medicinal treatment and indicating that immunological destruction of the amebas does not reach a high degree of perfection.

Emetin in the Treatment of Amebic Dysentery.—Naturally a large number of compounds have been advocated from time to time for the treatment of amebic dysentery by oral administration or rectal injection, but since the investigations of Vedder,[65] none have commanded as much attention as ipecac and one of its alkaloids, emetin. Recently an arsenical compound stovarsol, has been reported upon favorably but I shall discuss this and other arsenicals shortly in the treatment of dysentery.

Vedder has shown that ipecac and emetin possess an extremely high amebacidal activity *in vitro* for cultures of limax amebae and essentially similar results have been reported by Wherry,[66] Rogers,[67] Ryman and Wenyon,[68] Smith and myself[69] and others for ameba in the mucus of dysenteric discharges and the gingival pus of Rigg's disease. Dilutions of emetin as high as 1:100,000 are capable of killing the trophozoites in exposures of at least an hour at 38°C. although, as shown by Wherry, encysted parasites are much more resistant. In other words the life cycles of the endamebas exert an important influence and the difficulty of destroying the encysted parasites is largely responsible for the chronicity and relapses of amebic diseases, being analogous to the resistance offered by the sexual forms of the malarial plasmodia and suggesting that it may be possible to combine emetin with a dye or some other substance better able to penetrate the cysts.

Apparently Dale and Dobell[70] were the first investigators to undertake seriously an investigation of the curative properties of emetin in experimental amebic dysentery in kittens. They were however, unable to eradicate the infection and, furthermore, kittens receiving prophylactic doses of emetin were successfully infected. Mayer[71] reported similar results but in a more recent study by Sellards and Leiva,[72] employing cats and kittens infected with *E. histolytica*, good results were observed with rectal injections of emetin and quinin hydrochlorid while pafaverin and an extract of *Castela nicholsoni* (Chaparra amargosa) proved unsatisfactory.

Rogers[73] was first to use and recommend subcutaneous and rectal injections of emetin in the treatment of the amebic dysenteries of man, being led to give the drug a trial by the results of Vedder's investigations. He had long advocated the use of ipecac in amebic disease, and in 1907 demonstrated that the same drug would rapidly cure an amebic hepatitis in the presuppurative stage, and thus prevent the formation of amebic liver abscesses. Having been convinced of the action of emetin on amebas *in vitro*, Rogers

decided to use emetin hypodermically, and in 1912 he reported 2 cases of severe amebic dysentery and 1 case of acute hepatitis which were rapidly cured as the result of this treatment. Rogers later published further experiences with emetin in the treatment of 12 cases of amebic disease including cases of acute amebic dysentery, chronic amebic dysentery, acute hepatitis in which the formation of pus was prevented by the use of emetin, and of amebic abscesses of the liver cured by aspiration and injection of emetin.

Since then a rather extensive literature has accumulated upon the subject which need not here be reviewed in detail, except to state that enthusiasm with respect to the success of emetin has waxed and waned from year to year, indicating that much is yet to be learned. I believe, however, that it may be conservatively stated that the results of these *in vitro* and *in vivo* experiments show that emetin and quinin are strongly amebacidal. The problem is to bring them in contact with the amebas, as they occur in the deeper tissues of the intestinal wall in the amebic dysenteries. Rectal injections of emetin are apparently more efficacious than subcutaneous injections, at least in experimental infestments, but yet the latter have undoubtedly yielded good results in many amebic cases of dysentery of human subjects. Rectal injections of quinin, however, are apparently better than rectal injections of emetin, because much less toxic while definitely amebacidal and slightly bactericidal. In brief the value of emetin in amebic dysentery and amebic abscesses of the liver, may be summarized somewhat as follows:

1. Particularly good results are to be expected in amebic abscesses of the liver or other organs secondary to dysentery. Surgical evacuation may be required, but results show that emetin may completely cure small abscesses at least and that it should always be given even in surgical cases. Evidently emetin is better able to reach the endamebas in these abscesses than in the intestinal wall, but a more important reason, in my opinion, is that mixed bacterial infections are less likely to occur, which greatly complicate the treatment of the intestinal ulcers.

2. Emetin by subcutaneous injection, by oral administration or by rectal injection is much more efficacious in the early than in the later stages of dysentery. This is to be expected, because secondary bacterial infection is not so extensive, necrotic tissue harboring and protecting amebas has not been produced and chronic fibrosis has not developed, all of which may interfere with the amebacidal activity of the drug.

3. Emetin is but feebly amebacidal for encysted *E. histolytica* and may not completely remove them from the bowel. For this reason the parasites may be found in the intestinal contents for long periods of time after clinical cure and produce relapses. On the other hand, the endamebas found under such circumstances may not be *E. histolytica*, but the fact remains that, as long as amebas are found, the danger of relapse is present.

4. Unquestionably the subcutaneous injection of emetin has greatly improved our treatment of amebic dysentery and particularly of the acute early cases; at least, the relief of symptoms is oftentimes wonderful and much better than with the older forms of treatment, although Vedder's hope that the drug would accomplish in dysentery what arsphenamin has done in syphilis has not been realized in most cases. There is much yet to be learned, but I repeat that, in my opinion, the difficulty of curing amebic dysentery and particularly of chronic dysentery is principally due to three factors: (*a*) secondary bacterial infection; (*b*) the difficulty of bringing emetin or quinin in contact with the deeply situated amebas in *all* ulcers of the colon by enemas of these drugs, and (*c*) the fact that emetin is too toxic by all routes of administration to permit the administration of sufficient amounts over a

sufficiently long period of time to destroy all of the parasites, particularly the encysted ones, in some cases of amebic dysentery, although enough may be given to afford symptomatic relief and to hold the disease in long periods of check and latency. Furthermore, emetin is so low in bactericidal activity as to be without any effect upon the secondary bacterial infections of amebic dysentery and is likewise useless in the treatment of bacillary dysentery.

Arsphenamin, Neoarsphenamin and Stovarsol in the Treatment of Amebic Dysentery.—It is no wonder that arsphenamin and neoarsphenamin should have been employed in the treatment of amebic dysentery and especially when it was discovered that these arsenicals were poisonous for trypanosomes, spirochetes and other protozoa. Unfortunately however, no one appears to have used these drugs in the treatment of experimentally produced amebic dysentery, so that an opinion of their value must be based alone upon clinical results.

But quite a large number of clinical reports by Win,[74] Willets,[75] Gunn,[76] Chalmers and Archibald,[77] Ravaut and Charpin[78] and others indicate that intravenous injections of arsphenamin and neoarsphenamin have been apparently beneficial in the treatment of amebic dysentery and carriers. Lawson, of Roanoke, informs me that he has found rectal infusions of arsphenamin (0.6 gm. dissolved in warm water without neutralization) of great help and he has also administered this compound in salol coated pills.

But recently Garin and Lepine[79] have employed stovarsol in the treatment of amebic dysentery and its complications with considerable success. The plan was to administer 0.5 to 0.75 gm. daily in capsules during the first and third weeks while emetin was given during the second and fourth weeks of treatment. Johns and Jamison[80] have reported exceptionally good results in the treatment of 46 cases of amebic dysentery by the oral administration of 0.5 to 1 gm. (8 to 15 grains) of stovarsol each day for three to six days in succession. Clinically the cases ranged from the acute fulminating infections to the chronic varieties and the treatment was found especially advantageous in the treatment of such difficult cases as patients with severe cardiac lesions, with the toxemia of pregnancy and in very small children. No relapses were noted over a period of observation of about four months.

Methods for the Specific Treatment of Amebic Dysentery.—Naturally many different methods have been advocated for the treatment of amebic dysentery but it is not possible to lay down any definite plan suitable for all cases as each individual case is a problem by itself. The following plan is offered only in the way of a suggestion likely to be applicable for the treatment of most cases:

1. The patient should be confined to bed and a cathartic given, one half ounce of Epsom salt or castor oil being especially efficacious. Very careful nursing is required with particular care of the mouth and skin, bed-sores being especially likely to occur in chronic cases if the buttocks are repeatedly soiled with the bowel discharges. A nurse especially experienced in giving colonic irrigations should be employed. Due care should be exercised in the disinfection of the discharges and soiled bed-clothes; also in the cleansing and disinfection of the hands.

2. During the acute stages a liquid diet is required and a generous milk diet is especially suitable, although a soft diet is permissible for most cases, due care being taken in the selection and amounts of food to minimize the residue, abdominal distress, flatulence and incomplete digestion. Plenty of cool plain water, seltzer water or diluted ginger ale may be given, the latter especially if nausea is present. In severe cases with dehydration of the tis-

sues the intravenous injection of 500 c.c. of saline solution is sometimes required.

3. Stovarsol may be first tried in dose of 0.5 gm. (7½ grains) in a capsule twice a day for three to six days. If the acute symptoms subside the dose may be reduced to one capsule per day for an additional week or two followed by careful examination of the feces for ameba at intervals over several months.

4. If stovarsol is not used or fails, treatment with emetin, quinin and bismuth may be undertaken somewhat as follows: (1) A large daily dose of bismuth subgallate (60 grains) suspended in water or milk; (2) two daily doses of 1 grain of emetin bismuth iodid in lightly keratinized capsules; (3) one or two daily subcutaneous or intramuscular injections of emetin hydrochlorid in dose of ⅓ grain; (4) a daily high colonic irrigation of 1:1000 quinin hydrochlorid in comfortably hot starch water. An arsphenamin irrigation prepared by dissolving 0.6 gm. in 1000 c.c. of water or 1:500 protargol may be used occasionally, but emetin enemas should not be given in view of the amounts administered by ingestion and injection. In very severe cases threatened with gangrenous colitis, appendicostomy with irrigation of the colon with quinin solution or 1:500 collargol may be required. Colonic irrigations with a pint to a quart of kerosene have been used successfully by Dowling and others; due care must be taken against irritation of the skin.

5. After the acute symptoms have subsided and usually within 10 to 12 days, the injections of emetin may be stopped while continuing with the daily doses of emetin bismuth iodid by ingestion and the colonic irrigations of quinin for another week.

6. The heart and circulation must be carefully watched while emetin is being given; strophanthus or digitalis may be administered if required and 0.5 to 1.0 c.c. of adrenalin chlorid by intramuscular injection if collapse occurs or is threatened.

7. The bowels may require castor oil every two or three days, being best given with a few drops of tincture of opium in soft capsules. Tenesmus may require no further treatment, but if severe, 30 drops of a 20-per cent. alcoholic solution of benzyl benzoate may be given once or twice a day; sometimes hot turpentine stupes or a hot water bottle over the abdomen are required and the former especially if there is colic and flatulence. Morphin should be avoided.

8. The stools should be carefully examined at frequent intervals for *E. histolytica* and especially for the encysted forms. If these are absent the administration of emetin bismuth iodid may be stopped, as likewise the colonic irrigations, or the administrations gradually reduced.

If encysted parasites are found associated with slight symptoms, emetin bismuth iodid may be continued in dose of 1 grain twice a day; but as a general rule, it is inadvisable to give a total of more than 40 grains without a rest of one or two weeks, this being necessary to avoid cumulative toxic effects. Quinin in dose of 5 grains, or 6 ounces of infusion of *Chaparro amargosa* (bitter bush), may be given twice a day before meals as substitutes. It is sometimes very difficult to get rid of this residual infestment and occasionally the encysted ameba are found over long periods of time without apparent lesions or symptoms. It is always advisable, however, to continue medication, since relapses are likely to occur as long as parasites are present. Stovarsol or arsphenamin are worthy of trial.

9. *Relapses* are treated in much the same manner as the initial attack, the choice of drugs and doses being guided according to the severity of the

lesions. Colonic irrigations with 1:1000 quinin are especially indicated, substituted now and again with irrigations of arsphenamin solution. A few intravenous injections of neoarsphenamin in dose of 0.6 gm. may be given; aside from the possible amebacidal effects, the arsenic may aid in improving the blood and nutrition. The matter of diet and physical regimen are very important in relation to the correction of the asthemic and neurasthemic states so likely to be present. Stovarsol may be tried.

10. To prevent late hepatic abscesses, Stitt recommends the intramuscular injection of 1 grain of emetin hydrochlorid daily for two or three days in succession each month, until the feces have been free of parasites for several months at least, when the danger is largely removed.

11. The treatment of chronic amebic dysentery presents many difficulties and is always tedious and prolonged. Stovarsol is worthy of trial in dose of 0.5 gm. in capsules two or three times a day. Emetin bismuth iodid may be given in dose of 2 grains in salol coated pills twice a day for two weeks at a time, interrupted by one or two weeks in order to avoid cumulative toxic effects; during this time 6 to 8 ounces of an infusion of *Chaparro amargosa* (bitter bush) may be given by mouth, one half hour before meals.

High colonic irrigations are especially useful, one or two being given each day. For this purpose one or two quarts of 1:1000 solution of quinin, infusion of *Chaparro amargosa*, 1:1000 silver nitrate or 1:500 protartol, may be employed. For ulcers reachable through the protoscope or sigmoidoscope, applications of 25-per cent. silver nitrate may be made.

To the best of my knowledge, carbon tetrachlorid has not yet been employed for the treatment of acute and chronic amebic dysentery, but in view of the excellent results observed in the treatment of hook-worm infestments with this substance, it may be worthy of trial in dose of 2 or 3 c.c. a day for two or three days in succession.

Specific Treatment of Amebic Liver Abscess.—It would appear that ipecac and emetin are indicated in all other clinical types of amebic diseases and especially in amebic abscesses of the liver when the chance of preventing them by early specific treatment has been lost. By open surgical drainage alone the mortality has been in the neighborhood of 57 per cent. Rogers[81] has recently stated that sterile aspiration of the contents followed by injection of a 1:50 solution of quinin dihydrochlorid combined with large doses of ipecac by mouth or emetin by injection, has reduced the mortality in 111 cases to about 15 per cent. or less. Certainly it would appear advisable to employ either ipecac or emetin in all cases in combination with appropriate surgical measures.

Specific Treatment of Oral Amebiasis.—As shown by Barrett and Smith[82] and independently by Bass and Johns,[83] *Endameba gingivalis* (Gros) may be found in smears of the secretions of pyorrhaea dentalis and alveolaris and possibly play an important rôle in the production of infection of the gums and alveolar processes. These endamebae have also been found in apparently healthy mouths, and it is not now generally believed that they are the primary cause of gingival infections but that the parasites by their digging and migratory habits, may carry bacteria and spirochetes into the deeper tissues and thereby open up avenues of infection and prove very important as a secondary etiological factor in these conditions. Rivas[84] states that he has found in the secretions of a case of gingivitis, endamebae closely resembling *E. histolytica* and if this should be confirmed, a more intimate relationship between oral and intestinal amebiases and even of amebic dysentery may be established.

Whenever the gingival secretions show the presence of amebas it is well to employ measures for their removal along with such surgical measures as may be required and the local application of solutions of arsphenamin and bactericides for the treatment of the more important spirochetic and bacterial infections, already discussed and described in Chapters VIII and XXXI.

Smith and Barrett have recommended the local application of 0.5 to 1-per cent. solutions of emetin, care being taken to introduce the solution into the pockets by means of a syringe fitted with a small curved, blunt needle. Bass and Johns have advocated the daily subcutaneous injection of emetin hydrochlorid in dose of ½ grain for three to six days in succession but local applications would appear sufficient in most cases.

Chemotherapy of Balantidic Dysentery.—Somewhat similar to the lesions and symptoms of amebic dysentery are those occasional dysenteries caused by the ciliates *Balantidium coli* and *Balantidium minuta*. These parasites are especially found in hogs and while originally regarded by Malsmsten, Doflein and others as harmless commensals of man, the investigations of Strong[85] and Solowjew[86] have definitely proved that they may burrow into the mucosa of the colon and in conjunction with bacteria produce an ulcerative colitis with lesions and symptoms similar to those of amebic dysentery.

The parasites are found by examination of the stools and, while they may be present without apparent lesions or symptoms, yet their presence is fraught with danger. The parasites are capable of encystment like *E. histolytica*, which adds greatly to the difficulty of removing them by medicinal measures.

The disease is most common in the tropical and subtropical countries, but many cases have been reported in the United States. Glaessner[87] states that the parasites contain a hemolytic toxin analogous to the hemotoxins of other parasitic worms as described by Schwartz[88] and others. Abscesses of the liver have been described, due to the entrance of the parasites into the portal veins.

The treatment of balantidic dysentery is usually difficult and unsatisfactory. To the best of my knowledge, no systematic chemotherapeutic investigations have been made, probably because of the difficulty of reproducing the disease experimentally, although it is claimed that kittens and adult cats can be infested by colonic injections of fecal material containing the parasites. Dutcher[89] reports that intravenous injections of arsphenamin once a week for a series of 3 to 6 injections, have proved of great value; at least large numbers of parasites were discharged in the feces with prompt improvement of the anemia. Ipecac and emetin, so efficient in amebic dysentery, are apparently useless in this infestment; tartar emetic has likewise failed. Thymol has been apparently successful in some cases. Colonic irrigations with tannic acid, vinegar, kerosene, protargol, quinin, etc., may be helpful.

Greene and Scully have reported that simply placing the patient on a milk diet may be sufficient for recovery; apparently the milk produces a medium unfavorable for the parasites and at the same time furnishes fluid for the desiccated tissues while being without irritation for the inflamed intestinal mucosa. They give 2½ quarts of whole milk per day divided into small portions at regular intervals. After several days the milk is supplemented by the addition of one or two soft eggs and later, when the bowels become sluggish, by stewed fruits. Bismuth subnitrate is given during the first day or two to check the diarrhea.

If these simple measures fail it would appear worth while to inject arsphenamin or neoarsphenamin intravenously once a week. Salol coated pills of arsphenamin may also be given; thymol by stomach may prove

helpful as likewise capsules of 1 to 2 grains of methylene-blue. I believe one or two doses of 2 to 3 c.c. of carbon tetrachlorid by stomach may be worthy of trial; possibly colonic irrigations with this substance may be more efficacious although great care must be exercised against absorption of toxic amounts and the production of local irritation. Possibly colonic irrigations with 1:2000 solution of thymol may be useful, as they have been found efficacious for the removal of *Lamblia intestinalis*, another parasitic infestment shortly to be discussed. Colonic injections of kerosene may be tried; likewise irrigations with 1:1000 quinin, 1:3000 methylene-blue or 1:500 protargol.

Chemotherapy of Lambliasis or Giardiasis.—Infestment of the intestines with *Lamblia intestinalis* may produce no discernible lesions or symptoms, the presence of the parasites being detected by routine examination of the feces or duodenal contents (Lyon-Meltzer method). But as recently stated by Musgrave,[90] a chronic diarrhea with intermittent exacerbations, digestive disturbances and nervous symptoms may be produced, the infestment being particularly severe in children, quite common in the tropics, and variously designated as flagellate diarrhea, tropical diarrhea and monad diarrhea. The disease, however, is not confined to tropical countries, but is world-wide in distribution; I have seen several cases in Philadelphia during the past four years. It was particularly prevalent during the World War, being generally known as trench diarrhea.

Death rarely results from *Lamblia* infestment and consequently very little is known of the pathological changes, but available evidence indicates that the parasites may produce a catarrhal enteritis involving the small intestine and, either primarily or as secondary invaders, aggravate lesions of the intestine by other agents. The flagellate forms are limited to the small intestine except in diarrhea, when they are found in the colon and feces; the encysted forms are regularly found in the feces and may reach the liver with the production of abscesses. Musgrave states that he has encountered no instance of prolonged parasitism without symptoms, the characteristic symptom being a persistent diarrhea. Constipation, however, may be present rather than diarrhea and the parasites have been known to invade the gall-bladder; Kantor suggests that *Lamblia* cholecystitis may be a definite clinical entity. Abdominal pain of varying degree is usually present in all cases presenting symptoms.

A few systematic investigations in the chemotherapy of the disease have been made and especially with arsphenamin and neoarsphenamin. It is stated that mice may be experimentally infested by fresh human material; I have failed to successfully infest white rats and guinea-pigs. Kofoid, Boeck, Minnick and Rogers[91] state, however, that rats may be naturally infested with a species of *Giardia* quite similar to *Lamblia intestinalis* and they have found that massive doses of neoarsphenamin (4 to 8 times larger per kilogram than given to human beings) may stop cyst formation and bring about a disappearance of the parasites; ordinary single doses, however, were without effect. Yakimoff and his colleagues[92] had previously shown, in 1917, that mice experimentally infested with *Giardia* may be cured by injections of 1 c.c. of 1:300 to 1:1000 solutions of neoarsphenamin per 20 grams of weight corresponding to 0.005 to 0.015 gm. per kilo or 0.3 to 0.9 gm. for an adult of average weight.

Cade and Hollande,[93] however, failed to cure the infestment by administering arsphenamin by mouth; emetin subcutaneously, quinin sulphat and picric acid also failed. Goiffon and Roux,[94] Mantovani,[95] McGill,[96] Hollander[97] and others, also obtained indifferent results with arsphenamin and neoarsphenamin, but, as indicated by the reports of Carr and Chandler,[98] Kantor,[99] Reverdin and Chomé[100] and Simon,[101] the failure of arsphenamin and neoarsphenamin may have been due to the administration of too small doses; the latter also employed duodenal lavage with these arsenicals with success. Lyon and Swalm[110a] have recently reviewed the literature very carefully and state that intraduodenal injections of magnesium sulphat and 100 c.c. of 2 to 4 per cent. solutions of dimol may be effective for removing the parasites in the duodenum but without any effect upon those in

the biliary passages. Owing to the frequency of relapses they advise against drawing too hasty conclusions regarding any method of treatment. I would suggest the oral administration of carbon tetrachlorid or tetrachlorethylene as described on page 425, since these are known to be effective in the treatment of helminthiasis and especially hook-worm infestments.

Treatment with arsphenamin and neoarsphenamin, therefore, has offered more encouragement than any other drug; emetin, quinin, methylene-blue, tartar emetic, chloroform, sulphur, duodenal lavage with magnesium sulphat, Jutte's solution (0.9-per cent. sodium sulphate and sodium chlorid) and decinormal hydrochloric acid, etc., have all failed. Apparently the arsenic eliminated in the bile may be toxic for the parasites providing the concentration is sufficiently high and the following treatment may be found useful: (*a*) The intravenous injection of 0.6 to 0.9 gm. of neoarsphenamin every five days for 3 or 4 doses; (*b*) duodenal lavage with 100 c.c. of a 1-per cent. solution of neoarsphenamin if biliary drainage (Lyon-Meltzer method) is being given; (*c*) the internal administration of thymol in dose of 0.25 to 0.5 gm. (4 to 8 grains) in capsules with lactose and (*d*) enemas of 1:2000 solution of thymol, as recommended by Musgrave. While the parasites may disappear for a while, cure of the infestment cannot be regarded as accomplished until repeated and careful examinations of the feces or duodenal contents and bile have yielded negative results over a period of several months at least and preferably for a year or two. Stovarsol in dose of about 0.5 gm. per day has also been reported as yielding successful results in the treatment of *Lamblia* and *Trichomonas* infestments.

Chemotherapy of Trichomoniasis and Other Flagellate Infestments.—As is well known, the intestines and less frequently the stomach, mouth, respiratory tract, vagina, bladder and other organs may be infested by a species of the genus *Trichomonas*, characterized by no definite symptoms or by those due to local irritation. *Trichomonas intestinalis* is generally regarded as capable of producing diarrheal conditions in the tropics and respiratory and genito-urinary trichomoniasis may produce symptoms.

Nothing has been done, however, in the way of chemotherapeutic investigations in these infestments. The parasites are difficult to remove and vigorous measures are not required or indicated in the absence of symptoms. The same may be stated in relation to *tetramitiasis*, *chilomastixiasis*, *cercomoniasis* and *prowazekiasis;* if treatment is demanded, it may be along the lines discussed above for giardiasis.

Chemotherapy of Piroplasmosis or Babesiosis.—Before concluding this account of the chemotherapy of protozoan diseases, brief mention may be made of diseases of the lower animals caused by sporozoa belonging to the genus *Babesia*, or *Piroplasm*. The parasites infest the red blood corpuscles and three forms are especially well known, namely, *Babesia canis* (Piana and Galli-Vallerio), infesting dogs, *Babesia bigemina* (Smith and Kilbourne), infesting cattle and producing Texas cattle fever, and *Babesia bovis* (Babes) producing disease of cattle in Europe. Similar parasites have been found in the blood of sheep (*B. ovis*) and horses (*B. equi*).

These diseases, and especially babesia of dogs, are of great interest because they were very early the subject of chemotherapeutic investigations. Thus in 1909, Nuttal and Hadwen[102] found subcutaneous injections of 6 to 10 c.c. of 1-per cent. solutions of trypan-red and 5 to 10 c.c. of saturated solutions of trypan-blue, of some value in canine piroplasmosis; Theiler[103] and more recently Urbain[104] have likewise found trypan-blue of some value. Meyer[105] found trypan-red of no value in babesia of dogs (bilious fever or malignant jaundice), but trypan-blue demonstrated some degree of parasiticidal activity

in four animals. Nuttal and Graham-Smith have found quinin, tartar emetic, methylene-blue, etc., of no value. The pentavalent arsenicals, like atoxyl, sodium methyl arsonate, etc., have likewise proved without effect in babesia of both dogs and cattle; likewise arsacetin and sodium-para-amino phenylarsonate (soamin), and Nawrotski and others have stated that arsphenamin is without appreciable effect. Hutyra and Marek[106] have fully reviewed the literature on the treatment of piroplasmosis of dogs, horses, cattle and sheep, and those interested in these subjects are referred to this excellent work for a résumé of the subject up to 1922. I believe that stovarsol by mouth in dose of 0.02 gm. per kilo may be worthy of trial.

Chemotherapy of Coccidiosis.—At least three species of *Coccidia* have been found to be parasitic in man: (1) *Coccidium cuniculi* of rabbits; (2) *Coccidium hominis* and (3) *Coccidium bigemini.* The parasites invade the epithelial cells or villi of the intestine and the cells and ducts of the liver. Ottolenghi and Pabis[107] have infected young rabbits experimentally, and treated them with atoxyl, arsenophenylglycin and tartar emetic with negative results; otherwise, however, chemotherapeutic investigations do not appear to have been made although the subject urgently requires investigation. While *coccidiodal granuloma* is a different disease etiologically, brief mention may be made of it at this time. This infection is not of protozoon origin but is caused by a fungus or mold sometimes placed in the class of Ascomycetes. It is also known as *Blastomycosis coccidioides* and the disease has been described under the name of blastomycosis in some localities and sporotrichiosis in others. It is a striking fact that the majority of cases of coccidiodal granuloma have occurred in males living in California or in females who had lived in this state and particularly in the San Joaquin Valley. Nothing definite is known of the mode of transmission. In the majority of cases the atrium of infection seems to be the respiratory tract with the production of lesions in the lungs and glands resembling those of tuberculosis. In a few instances gastrointestinal, joint and skin lesions have occurred. Potassium iodid has been employed in treatment but with little or no success; to the best of my knowledge there has been developed so far no efficient method of treatment and the subject is deserving of investigation from the chemotherapeutic standpoint.

Chemotherapy of Schistosomiasis or Bilharziasis.—Since several of the parasitic trematodes are known to infest the blood-vessels and especially the portal venous system, whence the parasites and especially the eggs may be transported to the bladder, rectum, liver, lungs and other organs, many and early attempts have been made to destroy them by the administration of drugs by intravenous injection. Infestations with these parasitic trematodes may be designated with the general terms *schistosomiasis, bilharziasis or distomiasis.* The diseases so grouped are much more commonly encountered among the lower animals. But at least five of them occur in human beings in tropical and subtropical countries, where transmission is especially facilitated by unhygienic conditions, and where the high incidence and serious lesions and symptoms produced render them of great importance.

Naturally tartar emetic and other antimonyls have been widely employed because of the success attending their use in protozoan diseases, and since ipecac and especially emetin have proved so efficacious in amebiases. Arsphenamin and its substitutes have also been tried and, while a few of the earlier reports were encouraging, we now know that these arsenicals are without much effect upon the metazoa.

Clinical Varieties of Schistosomiasis.—Of the parasitic trematodes infesting the blood and blood-vessels of man and the lower animals or occasionally transported by the blood to organs or tissues not usually inhabited by them (the erratic trematodes), particular interest is attached to the following from the standpoint of specific therapy:

1. *Urinary schistosomiasis or bilharziasis* caused by the adult worm and eggs of *Schistosoma hæmatobium.* This parasite inhabits the portal venous system and its branches; also the small veins of the pelvis and rarely the vena cava and lungs. The females, however, are especially apt to migrate to the veins of the bladder where they and the eggs produce a disease characterized chiefly by periodic attacks of hematuria and cystitis.

2. *Intestinal schistosomiasis* or *bilharziasis,* caused by the adult worm and eggs of *Schistosoma mansoni.* The worms live in the portal vein but migrate to the intestines and especially the rectum in the capillaries of which they lay their eggs, which burst these vessels and escape into the mucosa, producing ulcers (schistosomic dysentery and fever), extensive granulomata (schistosomic tumors), etc.

3. *Hepatic distomiasis,* or *schistosomiasis japonica,* caused by the worm and eggs of *Schistosoma japonicum.* This disease is also known as "Katayama disease" or "dysenteric schistosomiasis." The adult parasite lives in the veins and arteries of the liver and in the mesenteric and pelvic veins. The eggs may be found in the liver, but more frequently in the lymph-glands of the pelvis and submucosa of the rectum, producing painful enlargements of the liver and spleen, with or without fever, urticarial and dysenteric lesions, anasarca, anemia and sometimes pulmonary and cerebral lesions.

4. *Pulmonary distomiasis* or *paragonimiasis,* caused by *Paragonimus westermani* (*ringeri*). The worm usually inhabits the lungs, but has been known to occur in the liver and other organs, when finding its way into the blood followed by general distribution produces a variety of clinical types of the disease, as the generalized, thoracic (pulmonary), abdominal (hepatic and intestinal) and cerebral types.

In addition to these, we may add the diseases produced by *Fasciola hepatica* and *Fasciola gigantica,* very common fluke infestations of the lower animals and especially found in the liver of sheep ("sheep-rot"), but occasionally found in man and especially in the lungs. In some manner, however, the parasites may gain access to the blood and have been found in the liver, the portal vein and its branches, the anterior tibial vein, subcutaneous tissue, the pharynx (producing a disease of North Lebanon called "halzoun"), etc. As shown by Christopherson,[108] the worms have a potential longevity in the tissues, displaying considerable energy throughout their (frequently) long life, traversing the portal vein between the liver and bladder or rectum. Their generative energy appears to be great, commencing early and continuing through life with the deposition of enormous numbers of eggs.

Experimental Schistosomiasis.—Bettencourt and Borges[109] report that adult *Schistosomas* appearing to be *S. hæmatobium,* have developed in mice experimentally infected with larvae and cercaria from snails taken from an area of endemic schistosomiasis in Portugal. This opens up a field for experimental chemotherapeutic investigation, although it would appear that the treatment of various types of bilharziasis has been satisfactorily solved by the administration of emetin hydrochlorid and tartar emetic. Fairley[110] has likewise experimentally infected animals with *S. spindalis* and reports that, while tartar emetic and urea stibamin failed to cure, absolutely specific cures were secured by intravenous injections of emetin hydrochlorid.

Specific Treatment of Bilharziasis.—While arsphenamin was at one time thought to be curative, the studies of Day and Richards,[111] Fulleborn and Werner[112] and others have since proved the arsenicals to be valueless.

But excellent and specific results have been observed following injections of emetin, Reguzis[113] stating that he was first to use the drug by intravenous injection in the treatment of *Bilharzia hematobium* infestments in Cairo, Egypt. Bonnet[114] and Tsykalos[115] have also reported excellent results with this drug, the latter injecting 0.02 to 0.010 gm. every two or three days for 6 injections followed by an intermission of six days and a second series of 6 injections in dose of 0.010 gm. No living parasites or living ova could be found after the fifteenth injection.

But apparently much better results have been secured by intravenous injections of tartar emetic, first introduced by Christopherson,[116] about 30 grains (0.180 gm.) in divided doses over a period of twenty-eight to thirty days, being sufficient. Pavy,[117] Quintini,[118] Petillo,[119] Tyau,[120] Libby,[121] Lasbrey and Coleman[122] and especially Cawston,[123] have reported

uniformly successful results with this compound. The treatment may be conducted as follows:

The injections (intravenous) are given on alternate days over a period of from fifteen to thirty days. The first dose is ½ grain (0.03 gm. of antimony and potassium tartrate dissolved in 90 minims (6 c.c.) of sterile physiologic sodium chlorid solution. The injection is repeated every other day and increased by ½ grain up to a maximum of 2½ grains (0.16 gm.) at a single sitting. In giving the larger doses, the total amount of an injection is made up to 10 c.c. with the diluting fluid. The total amount of the drug injected may amount to between 20 and 30 grains (from 1.3 to 2 gm.). Manson says that this amount may be shown by further observation to be too large. A rapid improvement occurs in the condition of the urine and after about 15 grains (1 gm.) has been injected all traces of blood usually disappear. Care must be taken that none of the solution escapes into the surrounding tissues, as an abscess may result. It is advisable to have the patient lie down for a few hours after an injection. A total of 10 grains (0.6 gm.) appears sufficient for children.

Emetin also has recently been given with apparent success. It is advocated for patients who are intolerant of antimony. It may be given intramuscularly, but whenever possible should be given intravenously in a 3-per cent. solution in sterile water, the dose being 2 c.c. containing 1 grain (0.06 gm.) of emetin hydrochlorid. The maximum single dose is 3 grains (0.2 gm.) for an adult. Generally one commences with ½ grain doses. A total of from 15 to 20 grains (1 to 1.3 gm.) may be given altogether.

Both drugs apparently act upon the ova especially since these show degenerative changes. They also probably affect the adult worms which gradually die off. The newer antimonyls, as employed by Randall in the treatment of granuloma inguinale, do not appear to have been used, but probably will yield equally good and even better results, since they appear to be less toxic than tartar emetic.

Specific Treatment of Fasciolopsis.—But the results observed in the treatment of fluke infestations have not been as good. Mauriac and Boyer[124] report the failure of emetin, tartar emetic, thymol, neoarsphenamin and male fern in the treatment of infestments of sheep and one human being. Since infestations of the liver and lungs are very frequent and especially in China, these diseases urgently call for systematic chemotherapeutic investigation.

Arsphenamin, Neoarsphenamin and Tartar Emetic in the Treatment of Trichinosis.—A very large number of drugs have been employed in the treatment of trichinosis, due to infestment of the muscles and other tissues, even of the spinal fluid, with *Trichina spiralis*. In 1914, Van Cott and Lintz[125] reported unsuccessful results in treatment with intravenous injections of arsphenamin, but McNerthney and McNerthney[126] have reported an apparently successful result from the intravenous injection of 0.6 gm. of neoarsphenamin. Alexander[127] likewise reports negative results in the treatment of three cases with injections of arsphenamin, but Nieto,[128] has recently reported uniform success with 2 or 3 injections of neoarsphenamin in dose of 0.45 gm. each. It would appear, therefore, that neoarsphenamin is at least worthy of trial and that two or three intravenous injections of 0.6 gm. each at intervals of five or six days may afford temporary relief if not complete cure. Grove[129] has recently reported the apparently successful treatment of a case by intravenous injections of freshly prepared 2 per cent. solutions of tartar emetic in dose of 3 to 4 c.c. for every 10 pounds of weight, gradually increased to 10 c.c., every second or third day.

Specific Treatment of Uncinariasis, Other Intestinal Infestments and Filariasis.—It is hardly necessary for me to discuss here the treatment of other forms of intestinal infestations with the various tape and round worms, although strictly speaking, this subject belongs to the domain of chemotherapy, since drugs are given for the purpose of poisoning the parasites without the absorption of sufficient amounts to produce toxic symptoms. I must mention, however, the excellent results being observed at present with the internal administration of carbon tetrachlorid in the treatment of uncinariasis (hook-worm disease) as discovered by Dr. Hall of the Bureau of Animal Industry of the U. S. Department of Agriculture.[130] He found that 0.3 c.c. of the drug for every kilogram of body weight sufficed to expel all the hookworms without subsequent purgation—a result that he had not been able to achieve with any other anthelmintic. Tests of the drug made in combination with thymol and with chenopodium produced equally favorable results. Monkeys, swine and horses were also treated, and postmortem examinations performed on these animals, months later, revealed no pathologic changes in the organs which could be attributed to carbon tetrachlorid. Dr. Hall himself took 3 c.c. of the drug without ill effects and Wells[131] has made a recent and thorough study of the absorption and excretion of carbon tetrachlorid from isolated intestinal loops of dogs.

Lambert[132] has reported particularly favorable results covering 50,000 treatments in Siwa, Fiji. The drug is practically tasteless but has a faint odor and produces a sensation of heat after being swallowed. He reports three fatalities due to impure compounds. Meyer and Pessoa[133] have likewise described histological changes in the liver and kidneys of dogs ascribed to over dosage and toxic effects. But the amounts now employed are regarded as quite harmless and if due care is exercised in the use of a pure compound, the danger of toxic effects is greatly reduced but not entirely removed. Lambert states that "the symptoms seemed to depend on the amount of absorption of the drug by the human host. The drug itself is a purge when taken in sufficient dosage on an empty stomach. The purgative action seemed to be diminished or lost, however, when the medicine was taken by one suffering from constipation, or when food has been taken just before or just after the drug. It is in these cases that symptoms of absorption occurred. These symptoms seem to be the same as those exhibited by a person recovering from chloroform anesthesia. The commonest is a dull headache, which may last for two or three days and is best relieved by free purging with magnesium sulphate. A few patients reported nausea and vomiting; many were sleepy for several hours after taking the medicine, and a few who followed directions to the letter said that they had suffered from headache. Since magnesium sulphate has been given as a routine measure headache symptoms have rarely been reported. With the cases under control, no symptoms are ever heard of."

The dose is about 0.2 c.c. (3 minims) for each year of age up to 15 years, when the adult dose of 2 or 3 c.c. is reached. The drug is placed on a tablespoon or in a small glass, covered with water and swallowed. When young children are also infested with *Ascaris*, it is well to use oil of chenopodium at the same time, Lambert adding one part of chenopodium to eleven parts of carbon tetrachlorid. The dose of the mixture remains at 3 minims (0.2 c.c.) for each year of age. This means $2\frac{3}{4}$ minims of tetrachlorid and $\frac{1}{4}$ minim of chenopodium for each year. He has now treated with this dosage several hundred persons, many of whom have had fairly severe *Ascaris* infections. The worms have all been removed dead. The mixture is not disagreeable to take, and children have not objected to it. The oil is held in a perfect solution in the tetrachlorid. The drugs may quite properly be given in the same proportions to adults in the accepted dosage of from 50 to 60 minims (3 to 4 c.c.).

Pregnant women may be treated with safety, but alcoholism increases the toxicity of the drug.

Lambert states that carbon tetrachlorid in the dosage here given is not so effective a vermifuge as oil or chenopodium for removing *Ascaris*, only

40 per cent. of the parasites being removed by the Fijian tests. The drug occasionally removes *Trichocephalus dispar* in small numbers, but it does not cure the disease. It seems, however, to remove *Oxyuris vermicularis* in large numbers. Recently Hall and Shillinger[133a] have reported that *tetrachlorethylene* may be slightly more effective than carbon tetrachlorid, the dose being from 2 to 3 c.c. for an adult.

Both carbon tetrachlorid and tetrachlorethylene have also been used with success for the removal of hookworms and round worms of dogs, being administered in doses of 3 to 16 minims in soluble gelatin capsules.

Practically nothing has been accomplished in the chemotherapy of *filariasis* of human beings and the lower animals. Arsphenamin and neoarsphenamin have occasionally yielded apparently encouraging results and may be worthy of trial, but atoxyl, arsacetin, arsenophenylglycin, emetin, tartar emetic, trypan-blue, etc., have yielded negative results and especially in the lower animals, the literature being summarized by Hutyra and Marek previously quoted. It would appear that intravenous injections of antimony thioglycollamide every two days is justifiable, the adult dose being 0.08 gm. dissolved in 20 c.c. of sterile distilled water. Recently Rose[134] has reported that intravenous injections of "Bayer 205" in dose of 1 gram dissolved in about 10 c.c. of sterile water, caused a sharp initial increase of embryos in the blood, followed by a pronounced fall, which was however, only temporary, though sometimes noted from time to time for 2 or 3 months. These effects appeared to be produced through some toxic action on the parent worm, and not on the embryos.

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PART VI

CHEMOTHERAPY OF DISEASES OF DOUBTFUL OR UNKNOWN ETIOLOGY

CHAPTER XXXIV

CHEMOTHERAPY OF THE ANEMIAS, MALIGNANT TUMORS AND OTHER DISEASES OF DOUBTFUL OR UNKNOWN ETIOLOGY OF MAN AND THE LOWER ANIMALS

There is scarcely a single acute disease of unknown or doubtful etiology in human beings in the treatment of which arsphenamin or its substitutes have not been tried; in the lower animals, these and the more toxic pentavalent compounds, like atoxyl, arsacetin and arsenophenylglycin, have also been tried, as well as a number of synthetic dyes. Aside from the possible parasiticidal effects, arsphenamin and neoarsphenamin may be expected to improve the metabolism and nutrition, at least temporarily and to a slight degree, and I am inclined to believe that these have been used as the basis for not a few reports of favorable curative effects, especially early reports shortly after the discovery of these compounds, which have not been generally confirmed. I suppose it is a rather common frailty of human nature for both patient and physician to note every apparent improvement with painstaking attention and enthusiasm whenever a new drug is under investigation and especially one that has promise of being "specific;" as a result some evidence of improvement is claimed with a too hasty publication of what may be nothing more than temporary improvement or favorable impressions.

Basis of Chemotherapeutic Investigations in Diseases of Doubtful or Unknown Etiology.—Upon first thought it would appear a useless expenditure of time and effort to endeavor to develop a chemotherapy for diseases of unknown or doubtful etiology. But this is far from being true in at least those acute diseases possessing all the characteristics of specific infections. Furthermore, some of these, like rabies, hog-cholera, small-pox, etc., occur in, or are readily transmissible to, the lower animals, thereby opening up a favorable avenue for laboratory research. It is true that chemotherapeutic investigations in such diseases are hampered, since the nature of the infectious agent is unknown, but they are not entirely hopeless or necessarily doomed to failure. In some instances the human disease has its counterpart in a disease of the lower animals; rabies is an example and possibly likewise the various malignant growths of rats and mice which are inoculable at will on a large scale. It must be admitted, however, that a disease of human beings not transferable to the lower animals is unfavorable for chemotherapeutic research, since clinical or empirical investigations in chemotherapy are seldom as satisfactory as those based upon preceding fundamental studies with the lower animals. But this applies equally well to those diseases of both

known and unknown etiology. In other words, we are still so ignorant of many of the fundamental principles of chemotherapy, insofar as the laws governing the relation being chemical constitution and the biological reactions of organotropism and parasitropism are concerned, that the whole subject is still largely a "hit or miss" proposition. But most progress is to be expected in those infectious diseases of known etiology transmissible to the lower animals and especially those of small size like the rabbit, guinea-pig, rat and mouse; next, with those of unknown or doubtful etiology but yet transmissible to the lower animals, and least with those of unknown etiology and apparently not transmissible to the lower animals. Even in the last-mentioned group, however, we can at least determine the important pharmacological and toxicological properties of compounds in the lower animals and use them empirically with the hope of accomplishing good while doing no harm.

While chemotherapeutic investigation is mainly applicable to those diseases of doubtful or unknown etiology, but known or strongly suspected of being infectious or contagious and thereby parasitic, yet the subject is not without interest in relation to the treatment of some known not to be infectious. In other words, a remedy may be employed for the *relief of symptoms*, even though it does not destroy or remove the cause. Arsphenamin, for example, has been used with some success in the treatment of anemias of apparently non-parasitic origin, pellagra, etc.; quinin and other alkaloids of cinchona bark are known to have a field of usefulness beyond the treatment of malaria, and emetin, tartar emetic, etc., are useful in diseases other than those of protozoal or metazoan origin. In other words, compounds easily qualifying for the honor of being classified as "specifics" may possess certain other general pharmacological properties of value in the relief of symptoms, even though they may not cure in the strict sense of eradicating the known or unknown cause of disease.

Chemotherapy of the Anemias.—Since Bramwell, in 1825, announced the beneficial use of arsenic in the treatment of pernicious anemia, Fowler's solution and other arsenical preparations have been constantly employed. With the discovery of atoxyl, arsacetin and other organic compounds, it was no wonder that these should have been used but never on a large scale because of their toxicity. Shortly after the discovery of arsphenamin, however, interest in this subject was greatly renewed and in 1911 Bramwell[1] reported on the treatment of two cases of pernicious anemia with intramuscular injections, followed in 1913 by a report on his experience with twelve cases, two of whom showed no change, three slight improvement and seven greatly benefited, one of whom later had a relapse. Since then a very large literature has accumulated upon the subject of the treatment of pernicious and other types of anemia with arsphenamin, neoarsphenamin and more recently, germanium dioxid, with many conflicting opinions of their value.

Mechanism of Therapeutic Activity of Arsphenamin and Its Substitutes in the Anemias.—If arsphenamin and its substitutes, or any other agent of reputed value in the treatment of anemia, are actually helpful, it must be in one or more of the following ways: (*a*) By the destruction of the parasitic agent producing the anemia, as *Sp. pallida* in syphilitic anemia; (*b*) by stimulating the bone marrow and other erythroblastic tissues; (*c*) by increasing the resistance of erythrocytes to hemolytic agents and (*d*) by promoting the elimination or neutralization of hemolytic or depressing agents, as the influence of arsphenamin in the correction of the depressing effects of mercurialization.

That arsphenamin and neoarsphenamin are capable of correcting the secondary anemia of syphilis is well known, and this is doubtless due to the destruction of *Sp. pallida* which apparently produces a hemotoxin and especially during the acute early stages of the disease. The same may be true, but to a much lesser extent, in the anemias caused by other microparasites, as the streptococcus and staphylococcus, if it is granted that these arsenicals possess some degree of bactericidal activity *in vivo*, which may be the case in streptococcus infection.

Whether or not arsphenamin and neoarsphenamin actually stimulate the bone marrow cannot be definitely stated, but I believe that such is probably the case in at least some instances of secondary anemia. Intravenous injections of rats and rabbits with arsphenamin, corresponding to 0.01 gm. per kilo, and neoarsphenamin, in dose of 0.02 gm. per kilo, have sometimes been followed by a slight leukocytosis with a slight drop in erythrocytes and hemoglobin during the following three hours, followed by a return to normal proportions within twelve to twenty-four hours. But I have never been able to increase definitely the erythrocytes above normal proportions in these animals by one to four injections of either drug in the doses mentioned, which corresponded to those given adult human beings of average weight; neither have I been able to elicit definite histological evidences of increased blood corpuscle formation in sections of the marrow of the long bones. It may be that arsphenamin and neoarsphenamin actually stimulate red blood corpuscles and hemoglobin production; indeed, I believe this does happen in some of the anemias over a brief period at least, but I have not been able to detect any increase in normal rabbits and rats or in human beings with approximately normal erythrocyte counts and hemoglobin estimations. That the leukocyte producing tissues are affected is indicated by the temporary leukocytosis sometimes following injections; also by those occasional accidents to these tissues with the production of asplastic anemias described in Chapter LI. Furthermore, I have observed a slight drop in the erythrocytes of syphilitic subjects within a few hours after the intravenous injection of 0.6 gm. arsphenamin in 150 c.c. alkaline solution, which may have been due to the effects of hemolysis, but I have not been able to detect a similar effect from the intravenous injection of 0.6 to 0.9 gm. of neoarsphenamin in 10 to 20 c.c. of sterile water.

In my experiments the repeated intravenous injection of arsphenamin and neoarsphenamin has had no influence upon increasing the resistance of the erythrocytes of rabbits, guinea-pigs and white rats to hypotonic saline and saponin solutions. But I have observed in three cases of severe syphilitic anemia a gradual return of the corpuscles to normal minimal and maximal resistance to hypotonic saline solutions, being analogous in this respect to the influence of arsenic upon non-specific resistance described by Hill[2] and others, but I am inclined to believe that these effects were due to the production of healthier erythrocytes as spirochetes were removed from the tissues, rather than to an actual increased resistance of the erythrocytes to hemolytic agents induced by the arsenic of arsphenamin or neoarsphenamin.

Just how arsenic, and especially arsphenamin and neoarsphenamin, tends to improve anemic states through an influence upon metabolism and elimination is difficult to state, but possibly this influence is exerted through the checking of oxidation with improvement in nutrition and weight. At least, the effects of arsphenamin and its substitutes are sometimes wonderful in cases of syphilis overdosed with mercury and presenting haggard and anemic states. Likewise, in the chloro-anemias of latent tuberculosis, constipation, cancer and in "indoor anemias," I have observed slight but definite improvement following small doses of neoarsphenamin by intravenous injection along with improved appetite and sometimes slight increase of weight and nutrition; but whether these effects are due to the influence of arsenic upon metabolism or to a stimulation of the blood-making organs cannot be stated. As mentioned in previous chapters it would appear that tryparsamid is even more efficacious than arsphenamin or neoarsphenamin in improving weight and nutrition, at least in cases of neurosyphilis.

Arsphenamin and Its Substitutes in the Treatment of Syphilitic Anemia.—Fournier in reviewing the anemias of syphilis states the disease may cause a diminution in the percentage of hemoglobin, a decrease in the number of erythrocytes and an increase in the number of leukocytes. He recognized five types of anemia in association with the disease: (1) the simple secondary anemia (the most common); (2) chorotic anemia; (3) chorotic anemia with leukocytosis; (4) leukemia and (5) syphilis imitating the picture of pernicious

anemia. He referred to the difficulty in some cases of deciding whether syphilis is the direct cause of the anemias or whether they are the result of associated symptoms, superimposed infections or misguided therapy. He quoted the old saying that "mercury is the iron of syphilitic anemia," but realized that while mercury may do good for a time that it cannot be given over a prolonged period without damage.

Foucar and Stokes[3] have reviewed the literature up to 1921 on the influence of arsphenamin and neoarsphenamin in the anemias associated with syphilis with the result that of nine clinical investigators six reported favorable results, one was opposed to treatment and two undecided or gave qualified opinions. Among investigators of the laboratory aspects of the problem of the influence of arsphenamin on the blood, five were in favor of the drug as a means of producing a rise in hemoglobin and erythrocytes and seven were against or gave qualified opinion. The general opinion appears to be that arsphenamin produces a transient destruction of erythrocytes followed by a rise of varying degree and permanence.

Foucar and Stokes have then reported upon the results of arsphenamin treatment of twenty-five cases of severe anemia associated with syphilis. Of these, thirteen cases presented the blood picture of pernicious anemia and twelve of severe secondary anemia. Five of the 13 patients with pernicious-like anemia improved under arsphenamin and 3 of these did not have demonstrable syphilis; 5 other cases became worse under arsphenamin and 2 of these had syphilis; of the 12 cases of secondary anemia, 5 improved under arsphenamin treatment, while, curiously, 5 with demonstrable evidences of syphilis, did not improve; 1 became worse. Mercury by inunction, used alone, produced an unfavorable reaction in 4 or 9 patients with anemias who had previously improved under arsphenamin, but all had primary anemia and subsequently showed evidences of a relapsing unfavorable course. Foucar and Stokes state, therefore, that in syphilis with anemia, mercury should be used with caution and especially if the picture suggests primary anemia of the pernicious type.

My experience has indicated that this complicated subject may be summarized somewhat as follows:

(1) The anemia of acute early syphilis is usually of the simple variety but may be progressive and quite severe during the first six months, when specific treatment has not been given. I have never seen the blood picture of pernicious anemia in these early cases but the so called chronic secondary anemia, which may develop in chronic syphilis, may present the blood changes of pernicious anemia and the true pernicious or specific cryptogenic hemolytic anemia may develop as a superimposed or independent disease.

Under treatment with arsphenamin, neoarsphenamin, sulpharsphenamin or mercury, the simple secondary anemia of early syphilis gradually improves; this is due to the destruction of *Sp. pallida*, the influence of arsenic upon oxidation and metabolism and possibly to a slight stimulation of erythroblastic tissues.

(2) The anemia of chronic syphilis is usually of the chlorotic type in which the hemoglobin is reduced out of proportion to erythrocytes. As a general rule, the blood improves under specific medication and especially when arsphenamin, neoarsphenamin, sulpharsphenamin or tryparsamid are administered; this is likewise ascribed to the destruction of *Sp. pallida*, but in larger degree to the influence of arsenic upon the retardation of oxidation and other metabolic changes.

(3) A chloro-anemia in chronic syphilis may be due to chronic tuberculosis, non-syphilitic nephritis, etc., or be a type of "constitutional anemia," which the patient has had almost as long as he or she can remember and certainly before syphilitic infection; this type of anemia usually improves under arsenical therapy but is subject to "ups and downs."

(4) The anemia of excessive mercurial treatment may be of the simple or chorotic type and improves very slowly spontaneously as mercury is eliminated; the arsenicals, however, tend to hasten restoration of the blood with a marked improvement in the general condition of the patient.

(5) Pernicious anemia as a *result of syphilis* is very rare. Foucar and Stokes state that apparently non-syphilitic cases of pernicious anemia may yield falsely positive Wassermann reactions, but my experience with 8 cases of pernicious anemia tested during the past four years by my new complement-fixation test, does not confirm this impression; in my opinion, pernicious anemia in the absence of syphilis does not give a positive complement-fixation reaction. It may be that syphilis alone can produce pernicious anemia; I have never seen a case in which this conclusion was warranted and doubt that such occurs. I have, however, seen several very severe anemias due to syphilis, presenting several of the blood changes of pernicious anemia including an increase of urobilin in the urine and feces. Doubtless, however, pernicious anemia may develop as a superimposed disease in syphilitics. While thorough treatment of the syphilis is indicated, yet the pernicious anemia is not likely to be cured by antisyphilitic treatment alone; as stated by Foucar and Stokes, blood transfusion should be a preliminary to arsphenamin when the hemoglobin is below 20 per cent. and reactions to arsphenamin injections must be carefully avoided since they may produce an alarming drop in hemoglobin.

Arsphenamin and Its Substitutes in the Treatment of Non-syphilitic Chloro-anemia.—In the chloro-anemias of chronic tuberculosis, furunculosis and other infections, intravenous injections of 0.1 to 0.2 gm. neoarsphenamin once a week for a series of injections, has sometimes yielded encouraging results in the way of an improvement in appetite, weight and slight progressive increases of hemoglobin and erythrocytes. Kall[4] and others have reported results of this kind and my experience has been that the results are usually as good as secured by subcutaneous injections of iron and arsenic without the disagreeable reactions sometimes accompanying the latter. Evidently these effects are mostly due to the influence of arsenic upon reduction of oxidation and metabolism rather than to any stimulation of the erythroblastic tissues or increased resistance of the erythrocytes to bacterial or other hemolytic agents.

But arsphenamin and its substitutes cannot be regarded as curative agents in a strict sense for the non-syphilitic chloro-anemias. These blood dyscrasias have a variety of causes and our chief efforts should be directed toward the detection and removal of these; when, however, it is believed that the administration of arsenic may hasten recovery I have no hesitation in recommending intravenous injections of small doses of neoarsphenamin (0.1 to 0.3 gm.) as a means for administering arsenic in an agreeable manner and in a form calculated to yield very much quicker and better results than follows the administration of inorganic arsenic by mouth. My usual plan in cases of this kind is to administer an injection once a week for six to ten weeks in succession while iron and strychnin are being administered daily by ingestion; the correction of constipation, which is usually present, receives particular emphasis, as likewise the correction of dietary and hygienic errors so commonly present.

Arsphenamin and Its Substitutes in the Treatment of Pernicious Anemia.—Naturally most interest has been placed in the treatment of pernicious anemia with arsphenamin and neoarsphenamin, owing to the fatal character of the disease. The etiology is still unknown but considerable data indicates that it may be due to an infection, probably not to a specific organism, but to a streptococcus or similar bacterial focal infection increasing erythrocytic destruction or hemolysis rather than retarding erythrocyte production, although asplastic changes in the bone marrow may be a terminal change. Hunter has particularly emphasized the importance of increased hemolysis

and especially in the portal distribution, as the primary change in pernicious anemia as indicated by the icteroid color of the patient, the distinctly yellow color of the plasma, polychromasia, fragmentation, shadowing and punctate granulation of the erythrocytes, as well as by enlargement of the spleen and the increase of urobilin in the urine and feces. It is not always possible to discover evidences of hemolysis, but the red, swollen and painful tongue, achlorhydria and progressive degeneration of the spinal cord along with the blood changes, are generally sufficient for diagnosis.

As stated above, it would appear that *Sp. pallida* may rarely produce a severe secondary anemia resembling the disease; at least, Leede,[5] Eason[6] and others have reported surprisingly good results from arsphenamin in cases of syphilis presenting the blood picture of pernicious anemia. But Winterfeld[7] has recently stated that there is no relation at all between syphilis and pernicious anemia, and when the two diseases co-exist, great care is required in treatment since arsphenamin may aggravate the anemia; in his opinion the anemia requires first consideration and by means of blood transfusion, with or without splenectomy, an effort should be first made to correct or improve it before the treatment of syphilis is undertaken.

As previously stated, Bramwell, in 1913 reported some success in the treatment of twelve cases of pernicious anemia with arsphenamin and since then Leede,[8] Steyser,[10] Boggs,[11] Lampe,[12] Lowrey,[13] Hobhouse[14] and numerous others have reported cases of the disease in non-syphilitic individuals benefited by the administration of arsphenamin and neoarsphenamin. To the best of my knowledge no cures have been attained but the compounds have appeared to favor the production of relapses of varying duration, which is about the best we can expect at present in the treatment of this dreaded disease. Similar results have also been observed with ascending doses of Fowler's solution by mouth pushed almost to the point of maximum toleration, as likewise with subcutaneous injections of sodium cacodylate. Iron, which may be so useful in the chloro-anemias, is of but slight or no value in this disease.

Just how the organic and inorganic arsenicals act in pernicious anemia is not known, but in those cases presumably caused by focal infections with hemolytic streptococci, I am inclined to believe that arsphenamin and neoarsphenamin may be of aid as direct bactericidal agents as discussed in Chapter VII, or indirectly, by stimulating leukocytosis and other non-specific bactericidal agencies. Possibly the arsenicals may stimulate the erythroblastic tissues, but they are evidently inferior to blood transfusions in this respect. Possibly they aid by reducing oxidation and otherwise improving metabolism and nutrition; in my experience they have not increased the resistance of the patient's corpuscles to hypotonic saline, saponin or serum hemolysins. But whatever the mechanism may be, these compounds are not able to cure the disease; whenever relapses of such long duration result as to amount to a clinical cure there is the suspicion of latent syphilis as the etiological factor.

When it is decided to use an organic arsenical in treatment, I believe that preference should be given to neoarsphenamin or sulpharsphenamin rather than to arsphenamin, because of the necessity for avoiding toxic reactions. I have not employed tryparsamid and do not know how it compares with the other two arsenicals in this regard.

My plan is to give a series of intravenous injections of neoarsphenamin in dose of 0.2 to 0.4 gm. dissolved in 10 c.c. of sterile water (solution is not hemolytic) at intervals of one to two weeks. When small transfusions of citrated blood are being given, it is permissible to add the neoarsphenamin

to the blood and inject both together. In my experience best results have followed a series of small transfusions (150 to 200 c.c.) of blood at intervals of one to two weeks along with intravenous injections of neoarsphenamin with the blood or by separate injections within a day or two after each transfusion. Some physicians are opposed to transfusions when evidences of hemolytic activity are present, but I believe in them at this stage of the disease, extraordinary care being taken, however, in the selection of donors to avoid reactions as far as possible and preference being given to direct methods of transfusion to avoid toxic citrate reactions. Weekly or bi-weekly examinations of the blood are made and the treatments stopped when the blood is progressively improving. These are followed, however, by occasional intramuscular injections of 0.2 to 0.3 gm. of sulpharsphenamin dissolved in 2 to 3 c.c. of sterile water, to continue the arsenical effects; also by the administration of Fowler's solution by mouth. Needless to state, a very careful search is made for foci of infection; when accessible, cultures are made and autogenous vaccines prepared and administered along with measures for the correction of other factors if present, as hypo-acidity of the gastric juice, constipation, dietary and hygienic errors. I have no opinion to offer upon the value of splenectomy; in two cases, however, presenting active signs of hemolysis, I have seen splenectomy apparently followed by prompt relapses lasting respectively eight months and a little over two years; in a third case, splenectomy was followed by progressive and rapid impoverishment of the blood and spinal cord degeneration with death about three months later. On the whole, however, I believe that splenectomy is indicated when the spleen is considerably enlarged and when the blood picture and urobilin tests show progressive hemolysis uncontrolled or checked by arsenic and blood transfusions.

Germanium Dioxid and Sodium Germanate in the Treatment of the Anemias. Within the past few years, considerable interest has been aroused in the possible value of germanium dioxid, and especially sodium germanate, in the treatment of pernicious and other anemias.

The existence of germanium, its atomic weight and chemical properties being foretold by Mendeleeff[15] in 1871 by the aid of the periodic law, it was not discovered until fifteen years later by Winkler[16] in the silver ore argyrodite at Freiberg, Germany. In 1922, Muller, having isolated a small amount of germanium in the Harrison Chemical Laboratories of the University of Pennsylvania, was interested in the matter of its toxicity as compared with arsenic, and in a study of this kind, Hammett, Muller and Nowrey,[17] in comparing the relative toxicity of germanium dioxid and arsenic trioxid for the mature albino rat, found that the former could be given subcutaneously in doses up to 0.180 gm. per kilo of body weight with no apparent toxic effects, but that the latter generally proved fatal in doses of 0.008 gm. per kilo of body weight.

A study of the blood by the same investigators[18] following subcutaneous injections, revealed a marked and sustained rise in the number of erythrocytes, the increase ranging from 1 million to 5 million erythrocytes per c.mm. This increase occurred, as a rule, within a week's time and after one or two injections. A greater response was always noted in those test animals with low initial counts and *vice versa*, thus suggesting a relationship between the degree of response and the original number of red blood cells present. In comparing the degree of response with the size of the dose, no evidence was obtained to show that the larger doses exhibited a greater stimulating effect than did the smaller ones. Histologic examinations of the bone marrow of the germanium-treated rats gave evidence of a marked increase of nucleated erythrocytes over the number present in the marrow sections of the controls. The circulating blood of the test animals likewise exhibited a greater number of young red cells than the blood of the control animals. No changes were noted in the white blood cells. Muller and Iszard[19] demonstrated a similar erythrocythemia following administration of germanium dioxid in the guinea-pig, rabbit, dog, and man. In the case of man, the dose was administered orally, and apparently absorption occurred very promptly when given in this manner. Germanium dioxid was found not to accumulate in the system, but readily eliminated through the kidneys and in small part through the alimentary tract. The lethal dose as calculated by Muller and Iszard was about 0.586 gm. per kilo of body

weight. In my toxicity tests employing white rats of 100 to 150 grams injected intravenously with a solution of germanium dioxid furnished by Dr. Muller, the maximum tolerated dose was found to be slightly higher than 0.510 gm. per kilogram. Tests for trypanocidal activity employed rats infected with *Tr. equiperdum* and treated with intravenous injections as described in Chapter X, according to the technic of my method, showed that doses as high as 0.510 gm. per kilo restrained trypanosome infections for a day or two, but were unable to sterilize the animals; spirocheticidal tests employing rabbits with acute testicular syphilitic lesions and treated intravenously with germanium dioxid, were without effect. It would appear, therefore, that the toxicity of this substance is very low, but that it is also practically devoid of trypanosomal, spirocheticidal and bactericidal properties.

Hammett and Nowrey were the first to report upon the administration of germanium dioxid in the treatment of various anemias; Leukins[21] and Schmitz[22] have reported favorable results but Alexander,[23] Stengel,[24] Garvin[25] and others have reported that germanium dioxid produced practically no increase of erythrocytes or hemoglobin. Nowrey,[26] working with normal rats, likewise found that injections of germanium dioxid in neutral solution showed little or no change in the erythrocyte counts and hemoglobin estimations, but that a solution of sodium germanate produced a marked increase in erythrocytes and hemoglobin.

The exact status and value of germanium in the treatment of the anemias cannot be stated, but it would appear that sterilized solutions of sodium germanate (pH 7.4) are to be preferred to germanium dioxid and may prove worthy of trial in dose of 0.025 to 0.050 gm. by subcutaneous injection for an adult of average weight or 0.010 to 0.025 gm. by intravenous injection. By oral administration much larger doses (0.250 gm.) are required, which greatly increases the expense involved in the treatment. It would appear that the results have been much better in secondary anemias than in pernicious anemia, but this is to be expected and it is doubtful if germanium is superior to arsenic in these cases. It is practically certain that germanium has not fulfilled the hopes and expectations originally expressed in the treatment of pernicious anemia; but it is not reasonable to expect success in this disease until more is known of the nature of the hemolytic agents and processes concerned.

Chemotherapy of Malignant Tumors.—Owing to the high incidence and mortality of malignant tumors it is not surprising that early and persistent attempts have been made to unravel the mystery of their cause and to develop empirically an efficient means for specific treatment.

The ease with which certain cancers and sarcomas of the lower animals are transmissible through an indefinite number of generations along with certain manifestations of natural and acquired immunity, has greatly stimulated efforts towards the discovery of a parasitic cause and numerous organisms have been described in this connection. Space does not permit me to review in detail the large amount of investigation devoted to the etiology of malignancy but the results would appear to warrant the following deductions of direct interest in relation to chemotherapy.

Malignant tumors of human beings and the lower animals have been produced by prolonged irritation and notably by certain tars, paraffin and soot. It is possible that chemical agents of these kinds produce fundamental changes in the cells enabling them finally to produce continuously and of their own accord, growth-stimulating substances which normal tissues elaborate only in response to cell damage, that is, in malignancy the cells acquire the property of producing continuously and in relatively large amounts the same sort of stimulating substance that adult tissues produce or release when injured, for exciting reparative processes. The converse of this mechanism however, is also a possibility, namely, that prolonged irritation

results in the cells producing substances capable of neutralizing physiological restraint upon cellular proliferation and activity. Since, however, chemical irritants of these kinds may be concerned in only a fraction of malignant growths of human beings, other irritants must be thought of and it is possible that a specific parasite or any one of a number of non specific organisms may produce toxic agents capable of acting as the same kind of irritant and bringing about this fundamental and transmissible change in cells resulting in the continuous production of a cell stimulating substance responsible for their rapid and riotous proliferation. On the other hand malignancy may be due to a combination of factors of these kinds as recently stated by Gye and Barnard,[27] namely, that malignancy is caused by a specific virus but that it is ineffective until a second substance, obtained from tumor cells, breaks down the cell defenses and enables the virus to infect. This second substance in turn may be a product of the cells from the effects of prolonged irritation. The experiments upon which this hypothesis is based were conducted largely with the Roux chicken sarcoma. But Harkins, Schamberg and myself[27a] have not been able to confirm this work. In our experiments when sufficient chloroform was added to the tumor emulsion to kill the virus in the preparation of "specific factor," we were unable to produce tumors by injecting it along with subcultures of the primary tumor culture. In other words we believe that the so called "specific factor" of Gye is an attenuated but living virus of the tumor, rather than a chemical substance from the cells.

How may chemotherapy influence such processes? If malignancy is due to a specific virus alone it may be possible to discover a specific destructive chemical agent for it and this would be the simplest possibility of all. If malignancy is not due to a specific organism but rather to a fundamental change in cells as a result of prolonged irritation, an effective chemotherapy would be called upon to destroy the cells producing the growth stimulating or activating substances. If the irritant producing these changes were purely of a chemical nature like tar or paraffin, chemotherapy could do nothing in removing it but if the irritant were being produced by a specific or non specific organism, an effective chemotherapy would be expected to serve a dual function, namely, the destruction of the organism producing the irritant and secondly, the destruction of the cells already changed by the irritant to produce the cell stimulating substance.

But before passing in brief review the results of a large amount of investigation in this field, it may be well first to clearly establish in our minds what should be the criteria by which they are to be judged. This is all the more important, since as so well expressed by Weil,[28] "the natural course of the disease is associated with such fluctuations that a sanguine therapeutist can gain some encouragement from even the most hopeless cases. Hence it follows that every mode of treatment has found adherents. The market is flooded with cancer drugs, and cancer charlatans flourish in the most highly educated communities. Unfortunately even well-trained, honest and reputable physicians have fallen victims of this fallacy, and have lent their names to the support of modes of treatment which in reality produce no determinable effect on the natural evolution of the disease." It was the desire to combat this unfortunate tendency which led Weil[29] to attempt to establish the criteria of therapeutic effects in cancer, which were summarized with the statement that the *demonstrable reduction in size of a tumor, of a kind not to be attributed to the natural processes of evolution of that tumor or of its associated lesions, is the one essential feature of effective therapeutic intervention.* To this I would add that the effects must be of a permanent character and influence

favorably not only the primary growth but the metastatic lesions as well. Furthermore, that the effects should be lasting and recurrences prevented since it is apparently so easy to produce such temporary effects as reduction of pain, cessation of discharge and the healing over of carcinomatous ulcers by the administration of various tissue extracts and serums producing non specific protein reactions oftentimes of temporary benefit to the patient and readily enough interpreted as specific results by both the patient and physician.

In other words, so many substances like various so-called specific sera, vaccines of cancer cells, tissue and other autolysates, etc., which I have listed elsewhere,[30] may temporarily influence the associated symptoms of malignant tumors and in exceptional cases even bring about temporary or permanent regressions, that great care is required in accepting any of them as truly curative of the disease. It is not surprising that colloidal shock reactions elicited by protein agents or the heavy metals may produce inflammatory reactions about the tumors with improvement in pain or hemorrhage, but these alone are not to be accepted as criteria of cure.

With these criteria clearly in mind we may now briefly review the results of chemotherapeutic investigations in malignant tumors of the lower animals and human beings. No one has seriously questioned the true malignant nature of the experimental cancers and sarcomas of the lower animals and if anything were discovered truly curative for their metastases in the internal organs, great hopes could be entertained for similar effects in the malignant tumors of human beings, although softening of the subcutaneous tumors alone would be of much less significance. Of course all such studies have been purely on the "hit or miss" fashion since the etiology of malignancy is still unsolved, but I believe it may be stated that most investigators have worked with the idea in mind that the cancer cell itself is the primary object for destruction rather than a hypothetical parasitic agent. This of course would require of a chemical agent an extreme degree of specific affinity for these cells in order to spare the normal cells, which they so closely resemble.

It may be here stated however, that a substance unable to favorably influence an adeno-carcinoma of the mouse or a sarcoma of the rat, may not be necessarily without effect upon cancer of the human being; there is in other words a limitation to the value of animal experiments. The experimental disease runs a very much more acute course than malignant disease in human beings which may readily enough mask therapeutic effects, just as a severe experimentally produced bacterial infection may mask the therapeutic effects of an anti-serum or chemotherapeutic agent. I believe therefore, that animal tests have a greater positive than negative value and if anything is discovered truly curative for a mouse cancer, great hopes could be entertained for it in the treatment of cancer of human beings.

Probably the first scientific investigation was undertaken by Wassermann and Keysser[31] in 1911 in the treatment of cancers of mice with selenium-eosin compounds, the idea being that selenium may prove selectively destructive for cancer cells when brought to them by the eosin. Some cures were claimed and the report attracted a great deal of attention, but few details were given, the promised follow-up report was not made, Keysser[32] later reported that by far the larger proportion of animals died during treatment in the stage of softening and Uhlenhuth,[33] Contanim[34] and others were unable to confirm the findings. In truth the softening of the subcutaneous tumors was probably caused by trauma from excessive handling and squeezing of the growths; at least it was shown by Keysser[35] himself that selenio-vanadium compounds were without influence upon the metastatic growths in the internal organs.

It had been pointed out by Carl Lewin[36] that the therapeutic results obtained from subcutaneous mouse tumors, however encouraging, could not be logically applied to the treatment of human cancers. The subcutaneous transplanted tumors, as is well known, are as a rule limited by a distinct capsule and show no tendency to infiltrative growth. In this particular they present a most striking difference when compared with human tumors. On the other hand, the metastases of mouse tumors in the internal organs present an infiltrative mode of growth and thus approximate very much more closely to the human type of tumors. Keysser, therefore, determined to test the therapeutic effectiveness of his compounds on tumors implanted in various organs, but the results with carcinoma, adeno-carcinoma and sarcoma were regularly and consistently negative. Furthermore, it was shown that mixtures of eosin-selenium-vanadium and a suspension of mouse tumor cells *in vitro* did not result in the destruction of the latter, as shown by production of tumors when injected into mice.

In the meantime Neuberg and Caspari[37] thought that complex organic compounds of the heavy metals like zinc, platinum, tin, selenium, copper, silver and cobald may exert a favorable influence upon malignant mouse tumors by undergoing a transformation in the tumors to colloid substances which in turn brought about a destruction of tumor cells by increasing autolysis by ferments. For the latter assumption there is absolutely no evidence; it is due simply to the influence of the colloidal theory. If one critically examines the data on which this theory is based, one is forced to the conclusion that it has practically no established claim to validity. If we grant that colloidal metals have been shown to stimulate autolysis in the test tube, the same fact must be admitted of metals in non-colloidal solution. Izar[38] thought that he cured some rat tumors by injections of colloidal sulphur; Lewin made a similar claim for various preparations of gold in mouse tumors and Werner and Szecsi[39] produced similar results with a combination of selenium-vanadium with cholin-borate.

Keysser carried out experiments along the lines inaugurated by Newberg, using a combination of glycocoll and copper. He also tested the combination of borcholin with selenium-vanadium used by Werner and Szecsi. He was able to confirm the fact that both of these substances produced an unmistakable therapeutic effect on subcutaneous tumors. On the other hand, they were absolutely without influence on the internal tumors. In this respect, therefore, they were entirely comparable with the eosin-selenium compound. The thoretical basis constructed by Newberg, which rests on the assumption that the metallic compounds stimulate autolytic processes in the tumors, was also subjected by Keysser to destructive criticism. Finally, Keysser showed that none of these therapeutic agents were effective even in the case of subcutaneous tumors, unless the latter had reached at least the size of cherry pits. If a therapeutic injection were made immediately after inoculation of the tumors, no effect was observed. The tumors grew exactly as in the controls, and the animals died in about the same period of time.

It is quite clearly established therefore, that none of the preparations of which the therapeutic effectiveness has hitherto been proclaimed exercise any direct influence on the life or development of malignant tumors of the lower animals. Under certain very definite and restricted conditions, however, they may produce certain changes in the tumors, and in a small proportion even cures. These results, however, were obtained only in the case of tumors which were subcutaneous in location, not smaller than a cherry pit in size and probably caused by constant palpation and examination of the subcutaneous tumors resulting in circulatory changes with hyperemia and hemorrhage.

These circulatory changes in turn may be responsible for the increased tendency of the injected substances to lodge in the tumors, thereby possibly increasing the tendency to autolysis which the circulatory changes have inaugurated. It is, of course, questionable whether this explanation can be regarded as final but enough has been done to show that the iodids and various dyes, injected intravenously, tend to accumulate to a greater extent in the necrotic portions of subcutaneous and internal tumors of mice and human beings, than in other tissues. These in turn may increase autolysis and thus explain the results observed up to the present time on a purely non-specific basis and without involving the questions of specific destructive affinity between chemical agents on one hand and cancer cells and hypothetical cancer virus on the other.

Of course clinicians were not slow in utilizing these observations for the treatment of human cancers and the various types of preparation employed may be satisfactorily grouped under four classes as follows: (1) The crystalline salts of selenium; (2) selenium in colloidal solution; (3) other metals in colloidal solution; and (4) compounds of metals with organic radicals.

These substances have been administered by injection or by the mouth. Before passing to a further consideration of this subject however, it may be well to recall the fact that in the experimental tumors of animals, no matter what preparation has been used, it has been possible to accomplish therapeutic effects only by the use of relatively enormous doses of the medicament; of doses, in fact, which were scarcely lower than the lethal doses. Certain experimenters have noted that smaller doses actually stimulated the growth of the tumors. In the second place, it has almost invariably been found necessary to administer the treatment intravenously, inasmuch as the other modes of administration failed of therapeutic effect. It is quite apparent therefore, that a procedure in human beings in any degree analogous to that pursued in animals may be dangerous or impossible.

The majority of those who have worked with selenium have used it in colloidal form, either preparing it themselves or employing one of the preparations put on the market by the pharmaceutical firms. The results obtained are fairly concordant. The intravenous injection of these preparations produced but slight disturbances. There was leukocytosis, a moderate rise of temperature, and not infrequently a chill. Otherwise, the substances seemed to possess no toxicity. The effects produced on the tumors have almost invariably been reported as encouraging while some have observed nothing from selenium other than the temporary improvement which is shown by almost all cancer cases on the application of any new therapeutic measure.

Colloidal silver and colloidal copper have likewise been tried with generally negative results. McClurg, Sweek, Lyon, Fleisher and Loeb[41] however, have observed that intravenous injections of cancerous mice with colloidal copper and casein compounds sometimes produced a primary inflammatory change about the tumors followed by a secondary reaction leading to a diminution in size of the tumors by shrinking of the tumor and in some instances to a healing over of carcinomatous ulcers.

Of course arsphenamin and neoarsphenamin have also been tried but with entirely negative results in the experimental tumors of rats and mice; a few human beings have been reported as cured but it is highly probable that the lesions were syphilitic rather than malignant.

In this brief survey I have not attempted to cover the enormous literature upon the subject and especially that relating to the treatment of malignant disease of human beings. Suffice to state that for almost every preparation

described as beneficial in experimental tumors of mice and rats the claim has been made, practically without exception, that it exercises a markedly beneficial effect on malignant disease in the human being. How are we to interpret these observations? How are we to explain the fact that they are the invariable accompaniment of the most diverse methods of treatment? As stated by Weil, when the various methods of treatment which have been discussed are judged by the criteria given above, "it is apparent that none of them can lay claim to therapeutic effectiveness. The modifications of the disease attributed to them are modifications which occur spontaneously in a very large proportion of cases as a result of the natural evolution of the disease process. This is a fact which cannot be too strongly emphasized. Owing unfortunately to the hopeless character of cancer, men are not prone to study with care all the lesser changes which the disease and the patient present under ordinary conditions; but when a cure is under investigation, the patient and his medical attendant note every apparent improvement with painstaking attention and enthusiasm. As a result, some evidence of improvement in treatment is entered on the books." I believe that the subject however, is worthy of further efforts and especially if it is finally shown that cancer is due to a specific organism or organisms; in the meantime a search for chemical agents capable of merely arresting the active proliferation of tumor cells is worth while.

In this connection most attention is being attracted at the present time by the intravenous injection of lead in colloidal suspension by Bell and his associates[41a]. Bell has observed the frequency of abortions among lead workers due to coagulation necrosis of the chorionic epithelium and on the basis of the theory that cancer is a reversion on the part of starving cells to the nutriment seeking proclivities of their ancestral types, the chorionic epithelium, various preparations of lead, copper, thallium and thorium have been tried in the treatment of hopeless cases of cancer in human beings. Some rather remarkable results have been observed at present following intravenous injections of a colloidal suspension of lead prepared by the "sparking process," and stabilized with small amounts of gelatin and calcium chlorid. The amounts injected however, have been near the lethal doses and the patients kept on the verge of lead poisoning. It is stated that improvement and apparent cures have followed in some cases and especially in cancers of vascular tissues when the injections have been used jointly with roentgen ray and surgical treatment. At this writing however, the full details of preparation of the lead and dosage have not been disclosed and it is too early to express a definite opinion of this method of treatment.

Chemotherapy of Rabies.—The uniformly fatal nature of rabies in the lower animals and human beings has led to a number of attempts to develop a chemotherapy for this dreaded disease but with almost entirely negative results.

In 1908, Friedberger and Sachs[42] tried thioglycollic acid and atoxyl by subcutaneous injection in the treatment of seventeen rabbits, experimentally infected, during the incubation period; in one the incubation period was slightly prolonged and in another symptoms did not develop. Intravenous injections of arsenophenylglycin apparently cured one rabbit, but these results were probably due to some fortuitous circumstances, since they could not be duplicated; indeed, arsenophenylglycin in dilutions 1:10 to 1:1,000,000 with the virus *in vitro* was found unable to kill the unknown germ as determined by subdural injections of a portion of each mixture into rabbits.

Naturally, after the discovery of salvarsan (arsphenamin), this compound was employed; but the reports of Marras,[43] v. Lumbusch,[44] Mijia,[45] Wesson and others have shown that intravenous injections of arsphenamin and neoarsphenamin, even in massive doses, have had no effect upon either experimental rabies of dogs, rabbits and guinea-pigs or upon the disease of human beings.

The only really encouraging results observed up to the present time were reported by Moon[46] in 1913 who found that three dogs experimentally infected with street virus and treated with large doses of quinin by mouth survived, while the controls succumbed. In a subsequent study, however, Moon[47] found that quinin by mouth and subcutaneous injection failed to be regularly effective as a cure or preventive of rabies in animals. Given in the later stages of the disease in two human beings, it was likewise ineffective. Moon believes, however, that quinin may retard the development of street rabies if given in the incubation period and that the organism is somewhat influenced, thereby opening up a "lead" for the development of chemotherapy for this disease. Frothingham and Halliday[48] and Krumniede and Mann,[49] however, have reported almost entirely negative results.

My associate, Dr. Malcolm J. Harkins, has recently repeated this work[50] with rabbits experimentally infected by subdural injections of fixed virus. Treatment was begun four hours after infection and continued throughout the period of incubation until symptoms developed. Quinin bisulphat and ethylhydrocuprein hydrochlorid, given in dose of 0.005 gm. per kilogram by daily intravenous injection, were without appreciable effects. Neoarsphenamin and sulpharsphenamin, given intravenously in the large dose of 0.15 gm. per kilogram four hours after infection and then every three days for a total of three doses, were without effect. Gentian-violet in dose of 0.010 gm. and mercurochrome in dose of 0.005 gm. per kilogram, by intravenous injection within twenty-four hours after infection followed by repeated injections at intervals of three days, were also without effect. Potassium tartrobismuthate, in dose of 0.05 to 0.125 gm. per kilogram by intramuscular injection, starting two hours after infection and repeated daily, was likewise without effect. Additional experiments were conducted by mixing 0.2 c.c. of an emulsion of rabies virus (fixed) with 0.2 c.c. of a 1:100 solution of neoarsphenamin; duplicate mixtures were prepared with 1:100 sulpharsphenamin, 1:250 flumerin, 1:500 flumerin, 1:500 mercurophen and saline solution (control). After keeping the mixtures in a water-bath at 38°C. for thirty minutes, rabbits were injected intracerebrally with 0.2 c.c. of each mixture; all developed rabies in six to seven days, showing that these compounds were without effect. These results are especially striking in view of the very high bactericidal activity of the mercurials (mercurophen and flumerin) and suggest to me that the germ of rabies must be well protected by location in large brain cells effectively resisting penetration by these substances.

Nothing of value, therefore, has been discovered for the treatment of rabies, and immunization with vaccine continues to be the best prophylactic measure. It is to be hoped, however, that efforts toward the development of an efficient chemotherapy will be continued, and especially since it is so easy to conduct laboratory experiments in rabies with dogs, rabbits or guinea-pigs. In this connection, I may also mention that Dr. Harkins has succeeded in producing the disease in white rats by intracerebral injections of 0.1 c.c. of emulsion of virus; the animals developed the dumb variety of the disease on or about the sixth day and succumbed on the seventh.

Arsphenamin and Neoarsphenamin in the Treatment of Chorea.—Milian[51] has particularly advocated the treatment of this disease with arsphenamin and neoarsphenamin, believing that a large proportion of cases are due to congenital syphilis, but in a recent thorough study of a series of cases along with an extensive review of the literature, Morse and Floyd[52] arrived at the conclusion that, while syphilis may be occasionally found in cases of chorea, it bore no essential etiological relationship to the disease. Their study included very careful blood cultures, blood Wassermann reactions and examinations of the spinal fluid, and they believe that the results show an intimate relationship between chorea, rheumatism and endocarditis indicating that chorea is probably caused by a group of organisms apparently entering through the tonsils or some other focus of infection. While neoarsphenamin, arsphenamin or sulpharsphenamin may be worthy of trial and especially since arsenic is of known benefit in many cases of the disease, it cannot be stated that these compounds possess specific curative properties.

Arsphenamin in the Treatment of Pemphigus, Pellagra, Warts, Herpes Zoster, Verruga Peruviana, Sprue and Small Pox.—Owing to the highly fatal character of *pemphigus* (vulgaris and foliaceus), it is not surprising that numerous and early attempts in treatment were made with intravenous injections of arsphenamin by Sutton,[53] Eschweiler,[54] Merz,[55] Stumpke,[56]

Frieboes,[57] Wolfe[58] and others. In some instances beneficial results were claimed, but it is possible that these were confused with the temporary remissions of the disease or that mistakes in diagnosis were made. I have not myself seen any benefit from intravenous injections of neoarsphenamin, except such slight improvement as may be expected from the effects of the drug on metabolism. I am convinced that the organic arsenicals do not possess a specific action in pemphigus, which, according to recent investigations of Eberson, may be a bacterial infection. My colleagues Greenbaum and Schamberg, have noted temporary improvement following intravenous injections of mercurochrome.

Siemens[59] has recently reported upon the treatment of 34 cases of *warts* by injecting into them 0.15 c.c. of 1:15 to 1:40 dilutions of arsphenamin in saline solution. The injections produced slight pain in most cases, but is reported to have cured a number in from ten days to four months. The results are interesting in view of the work of Wile and his colleagues, showing that warts are caused by a filterable virus; also, in view of the successful results reported in the treatment of *verruca plana* with arsenic. Lindsay[60] believes that there are many cases of warts which the intravenous injection of neoarsphenamin is justifiable, in the hope of accomplishing by intensive arsenical treatment what may not be possible with milder forms of arsenic.

Since various types of *herpes*, including herpes zoster, are now known to be caused by a filterable virus, the subject of chemotherapy in this disease becomes one of considerable interest and especially in view of the ease by which lesions may be produced experimentally on the corneæ of rabbits, guinea-pigs and white rats. Grebb[61] has reported the apparently successful treatment of 2 cases of herpes zoster with arsphenamin, but it may be that these effects were due to natural remissions. I have not myself observed any curative effects in herpes zoster to be ascribed to neoarsphenamin administered by intravenous injection.

With the assistance of Dr. Harkins, I have produced experimental herpetic infections of the brains of rabbits and the following compounds were injected intravenously within four hours after inoculation and repeated every two days in these doses per kilogram of weight: arsphenamin, 0.010 gm.; neoarsphenamin, 0.015 gm.; mercurochrome, 0.003 gm.; gentian-violet, 0.005 gm.; quinin bisulphat, 0.005 gm. and ethylhydrocuprein hydrochlorid, 0.005 gm. Untreated controls were included, but none of these compounds appeared to retard the disease beyond the usual variations shown by the controls; neither were curative effects elicited by the treatment of rabbits with lesions fully developed.

The etiology of *verruga peruviana* is still unknown, but many features of the disease indicate that it may be caused by a parasitic protozoon. Hypodermic injections of arsenic have been recommended and Castellani states that arsphenamin and neoarsphenamin are worthy of trial, but I do not know of any case reports showing that these compounds possess curative activity.

The etiology of *sprue* is still undecided, but evidence at present indicates that the *Monilia* described by Kohlbrugge may be the cause or at least an important secondary infection. Castellani and Low have described several species of *Monilias* which they believe may cause certain important symptoms, such as frothiness of the feces, but they are not inclined to consider these organisms as the primary cause of the malady. Smith,[62] however, has recently stated that experiments support Ashford's theory[63] of the specific etiology of sprue as due to *Monilia psilosis* by the production in guinea-pigs of a chronic moniliasis with gastro-intestinal lesions roughly comparable to

those found in man. The development of the lesions, however, was rarely noted except in animals fed on a diet partially deficient in vitamins, especially the antiscorbutic vitamins. This favors the view that a deficient diet, or diminished resistance, such as is common following long residence in the tropics, associated frequently with gastro-intestinal infections such as amebic dysentery, are contributory factors in its production.

Nothing in the form of specific chemotherapy has been developed for the disease, although it would appear that neoarsphenamin, emetin and tartar emetic may be worthy of trial on account of their general protozoicidal properties.

Arsphenamin has also been used by Lenzman[64] and others in the treatment of *small-pox* with apparently some benefit; also by Cranston[65] in the treatment of *pellagra*. But it is likely that the beneficial results reported have been due to the well-known general "tonic" effects of the arsenic rather than to specific parasiticidal action.

Chemotherapy of Contagious Pleuropneumonia, Equine Influenza, Foot and Mouth Disease, and Other Diseases of the Lower Animals.—Naturally many of the diseases of the lower animals of doubtful or unknown etiology have likewise been treated with arsphenamin, neoarsphenamin and other compounds, with the hope that good therapeutic results might be obtained, while at least no harm was being done. In several of these diseases really good and encouraging results have been reported and I shall here consider a few of them, with which I have some familiarity, although I am not equipped by experience to consider all the diseases of the lower animals in which chemotherapy has been tried with varying success. A very complete account of these, however, may be found in the treatise by Hutyra and Marek on the "Spezielle Pathologie und Therapie der Haustiere."[66]

The etiology of *contagious pleuro-pneumonia* of horses is still undecided, both *Streptococcus pyogenes equi* and *Bacillus (bipolaris) equisepticus* being regarded by some of the etiological agents. Arsphenamin and neoarsphenamin, however, have apparently yielded excellent results in treatment by intravenous injection. The dose of both is the same as for human beings, namely, 0.010 gm. of arsphenamin and 0.015 gm. of neoarsphenamin, per kilogram of weight. This amounts to about 3 gm. or arsphenamin and 4 gm. of neoarsphenamin for a large horse of ordinary weight and 2 gm. and 3 gm. respectively for smaller animals. Neoarsphenamin is probably to be preferred, because of lesser toxicity and greater ease of administration; 2 or 3 gm. are dissolved in 50 to 60 c.c. of sterile distilled water and injected slowly with a syringe. Arsphenamin is dissolved in at least 400 to 600 c.c. of sterile water and alkalinized, as described in Chapter LXII, before injection.

Practitioners are not agreed on the best time for injection, some stating that treatment should be given in the first day and others advising waiting to the fourth day, but the majority advise early treatment and not later than the third day after the onset of symptoms.

Toxic reactions may follow the administration of either drug and especially arsphenamin. In exceptional instances collapse has occurred with death of the animal, but in the great majority of cases the reaction disappears within four to six hours after injection with prompt amelioration of the fever and other symptoms of the disease.

Gordsjalkowsky,[67] in 1912, reported excellent success in the treatment of 35 cases occurring in a regiment of cavalry in Russia, with intravenous injections of 2 gm. of arsphenamin; one dose was ordinarily sufficient. Rummel has employed atoxyl but without success. Since then a very large literature

has accumulated on the subject and while it cannot be stated that neoarsphenamin is an absolute specific for the disease, it has been demonstrated that the drug produces a crisis, greatly shortens the duration and reduces the mortality from 5 to 20 per cent. to less than ½ per cent. During the World War both arsphenamin and, especially, neoarsphenamin, were used with great success and now that the costs of arsphenamin and neoarsphenamin have been greatly reduced, either compound may be more freely employed for the treatment of this dreaded disease and especially in epidemics among valuable animals.

Influenza of horses is regarded as an infection caused by an unknown filterable virus. Various drugs have been used with indifferent success, as 25 to 50 c.c. of 1:1000 methylene-blue mixed with the food and injections of atoxyl, arsenophenylglycin and other organic arsenical compounds. Arsphenamin and neoarsphenamin by intravenous injection, in dose of 0.010 to 0.015 gm. per kilogram respectively, appear, however, to have yielded very good results as reported by Gohre,[68] Stange and Szulewski,[69] v. Capelle[70] and others.

Foot and mouth disease, so prevalent and destructive among cattle in all parts of the world, is likewise believed to be caused by a filterable virus. Arsenophenylglycin, atoxyl and other pentavalent arsenicals have been employed in treatment without appreciable results, but arsphenamin and neoarsphenamin have apparently proven more efficacious in the way of shortening the course of the disease and reducing the mortality. Kroncke,[71] for example, has used intravenous injections of silver arsphenamin, starting with such a small dose as 0.1 gm. for an animal of average size. More than two injections were not required; the lesions were treated locally with chromic acid or calcium chlorid. Arsphenamin or neoarsphenamin may be used instead of silver arsphenamin and are apparently worth trying in the treatment of valuable animals, being given in dose of 0.010 gm. per kilogram or about 2 gm. for the average animal. Pullman[72] has recently stated that intravenous injections of trypaflavin are sometimes effective.

Darrow[73] has recently reported successful results in the treatment of *contagious lymphangitis* with neoarsphenamin.

Graybill[74] has tried ipecac in the treatment of *black-head* in turkeys, but with negative results.

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PART VII

INFECTION, IMMUNITY AND PATHOLOGY OF SYPHILIS IN RELATION TO TREATMENT

CHAPTER XXXV

MECHANISM OF INFECTION WITH SPIROCHÆTA PALLIDA AND THE PATHOGENESIS OF SYPHILIS IN RELATION TO TREATMENT

The etiological agent of syphilis is now so universally accepted as the parasite discovered by Schaudinn in 1905 and named by him and Hoffmann[1] as *Spirochæta pallida*, that this subject does not here require discussion. A few months later Schaudinn for biological reasons renamed the organism as the *Spironema pallidum*, but this name not being permissible under the rules of zoological nomenclature, was again changed by him on October 26, 1905, to *Treponema pallidum*. Since then both terms have been employed.

Of considerable interest in this connection however, is the possibility of the spirochete of syphilis having been first seen and briefly described by Donne[1a] in 1837, in a study of the chemistry and cells of the vaginal secretions. Certainly he appears to have seen spiral-shaped organisms in the secretions of syphilitic ulcers of the female genitalia and in one case of leg ulcer, which he called "animalcules," and states were first described by Müller under the name "*vibrio lineola*." Of course he may have seen saprophytic spirochetes in the secretions of these parts but states that the vibrios were not found in non syphilitic women and a perusal of his little book leaves one with the impression that he actually saw the spirochete of syphilis, produced the disease experimentally by inoculations with it and was himself convinced that the organism bore an important relation to venereal infection and especially to the etiology of the chancre. Indeed his work was so thorough that one is surprised that this organism failed to excite more interest in relation to the etiology of syphilis.

At the present time *Spirochæta pallida* is regarded by some as a bacterium, by others as a protozoon and by a few as belonging to the domain of Protista, i.e., to neither the plant nor the animal kingdom. The subject is not without significance and importance in relation to the science of chemotherapy, but the majority of zoologists now appear to be convinced that the organism is a protozoon, i.e., belongs to the animal rather than the vegetable kingdom.

Terminology: Spirochæta pallida vs. Treponema pallidum.—Most German and English writers have used the name *Spirochæta pallida;* French authors commonly employ at present the terms treponemes and *Treponema pallidum*. In the United States both terms are employed, the former, however, more commonly than the latter. Pusey[2] states that the term *Spirochæta pallida* is to be preferred, since it is best known, mostly employed and

hardly worth while abandoning in favor of *Treponema pallidum* until the biological evidence in favor of the latter term becomes absolutely conclusive. Stiles[3] has summarized various reasons for the use of the term *Treponema pallidum*, but in view of the difficulties and lack of unanimity in the taxonomy of the subject, I decided to employ the term *Spirochæta pallida* throughout this book with the understanding that it is the same organism known as *Treponema pallidum*.

Possibility of a Granular Stage in the Life Cycle of Spirochæta Pallida.—The physical characters of *Spirochæta pallida* are likewise well known but from the standpoint of the prenatal or congenital transmission of syphilis the question, whether or not the spirochetes have a morphological stage other than the well-known spiral or cork screw shape becomes of considerable interest and importance. For example, clinical experience indicates that the paternal transmission of syphilis may take place, that is, that the organism occurs in the semen and either enters the conceptional spermatozoon or that both parasite and spermatozoon separately penetrate the ovum. Owing, however, to the large size of *Spirochæta pallida* many have held that for physical reasons it is impossible or at least improbable, that *Spirochæta pallida* infects a spermatozoon, although it may invade the ovum.

Balfour[4] states that *Spirochæta gallinarum* of Sudanese fowl spirochetosis has a granular as well as a spiral stage and Leishman,[5] Blanc,[6] Fantham,[7] Nuttall,[8] and Hindle[9] also entertain the belief that *Spirochæta gallinarum* and *Spirochæta duttoni* adopt a granular form under certain conditions, and that a spiral form can develop from these granules which have been variously called coccoid bodies, infective granules, or spores. Hindle observed in histological studies of ticks that they undergo segmentation, suggesting a process of sporulation. Todd and Wolbach[10] maintain that these granules are not specific and may be found in non infected ticks but Fantham points out that the granules of normal ticks are not identical with the coccoid bodies of infected ticks.

Of interest in this connection are likewise the investigations of Meirowsky,[11] who has described buds along *Spirochæta pallida* in cultures which he believes are concerned in the processes of division and multiplication. It is possible that these bodies are artefacts, but I have seen them very distinctly in cultures of *Sp. pallida* prepared in my laboratory by Miss Clara Kast and they are of much interest in relation to a possible resting stage of *Sp. pallida*. Noguchi[12] has also described peculiar, highly refractile, round bodies which is very often found attached somewhere along the side of the body of the organism.

Since 1915, McDonagh[13] has suggested that the spiral form was only the male phase of a protozoön and that the life cycle of *Spirochæta pallida* commences with a spore or sporozoite which enters a connective tissue or endothelial cell as its host followed by a process of budding giving rise to several bodies, which later become differentiated into male and female elements. McDonagh has accordingly designated the parasite of syphilis as *Leukocytozoön syphilidis* and while his work lacks general confirmation, yet when considered along with the investigations and observations briefly summarized above, is deserving of further study.

It is well known that the syphilitic testicle of a rabbit may show no spirochetes when the secretions secured by puncture are carefully and repeatedly examined with the dark field method during the resting stage of the cycle of orchitis in this animal; yet inoculation of an emulsion of this tissue into the testicles of fresh animals may result in infection although the percentage of "takes" is not as high as when inoculation is done with tissue containing numerous actively motile organisms. Of course, there is always the possibility in tests of this kind of overlooking the presence of a few spirochetes since it is obviously impossible to include every bit by dark field microscopic examination. Filtration of emulsions of such latent and of actively syphilitic tissue followed by injection of the filtrates into the testicles of rabbits have yielded negative results which speaks against a granular stage of *Spirochæta pallida* to the extent of a filter passing virus being produced; of interest in this connection, however, is the report of Artzt and Kerl[14] who found the secretions of erosive lesions of three patients free of spirochetes and yet infectious for rabbits, producing testicular lesions upon inoculation.

These observations show, therefore, that *Spirochæta pallida* may be but one phase in the cycle of development of the parasite of syphilis and that there may be a spore or granular stage which we have no means at present of recognizing. If such were proved to be the case, it is possible that the organism may be in the latent lesions which, when examined histologically, shows no spirochetes. Likewise a granular stage would afford a ready means for explaining the seminal transmission of syphilis as also maternal or placental transmission. The subject is not without interest likewise, in connection with chemotherapy and especially in relation to the treatment of the so-called "Wassermann-fast" cases of syphilis.

Mechanism of Primary Infection in Acquired Syphilis.—Syphilis is always acquired from human beings, either by direct or indirect contact, since there are no natural intermediate hosts or carriers of *Spirochæta pallida* among the lower animals. In the majority of instances infections occurs by direct contact with open lesions and their secretions containing *Sp. pallida.* These lesions are usually situated about the genital organs, the lips or in the mouth.

Infection may occur by intermediate contact, but in practice such instances are decidedly uncommon. Eating utensils and drinking cups used immediately after a syphilitic with open lesions in the mouth and contaminated with saliva are most dangerous. *Spirochæta pallida* dies quickly when the secretions become cold and dried and infections from toilet seats and towels are unlikely and rare in actual practice. An individual may accidentally and unknowingly become an intermediate carrier; for example, Schamberg has recorded an instance where a young man contracted syphilis of the lips by kissing a girl, who in turn had been kissed a short time previously by a syphilitic male. Practically any part of the skin or a mucous membrane is susceptible to infection with *Spirochæta pallida.* In over 90 per cent. of cases infection occurs about the genital organs. Extragenital infections most commonly occur about the lips, mouth and fingers, although infection may occur in the eye, nose and practically any part of the body. The scalp is probably less susceptible than non-hairy portions of the skin and the same is true of the rabbit, which is infected with difficulty except in the eye, scrotum and testicles. It is commonly stated that extragenital lesions are less common in the negro; for example, Hazen[15] reports that in over 3000 cases of syphilis seen by him in the colored race, only three presented extragenital lesions and it may be that in this race there are agencies in the skin affording some protection against *Sp. pallida,* although this is certainly not true of the mucous membranes of the genital organs of either sex. The negro in his sexual relations is less given to amatory practices and this may account for the lesser frequency of extragenital infections in this race. Reasoner,[16] however, suggests that the chancre is a tissue reaction of defense and that in the negro this reaction or defense to initial infection is but poorly developed outside of the genitalia and that extragenital infections may occur more frequently but are not discovered.

Infection in syphilis is ordinarily the result of contact with lesions and secretions containing *Spirochaeta pallida.* The lesion may be so small, however, as may easily escape detection and particularly when it occurs about the genital organs of women. Syphilitic men with no open lesions are still infectious by way of their semen and women are commonly infected in this way; in these cases *Sp. pallida* may be derived from the testicles which are so commonly infected in men and if such is the case, these testicular lesions are potentially open sources of infection.

May men be infected by sexual intercourse with women presenting no demonstrable open lesions about the genitalia? In other words, is the

woman presenting no demonstrable lesions or symptoms of syphilis other than a strongly positive Wassermann reaction infectious? Of course, it is well known that under these conditions she may transmit the disease to a fetus in the uterus, but is she infectious by ordinary sexual contact? In practice instances of such infections apparently occur, but it is almost impossible to be sure that a minute open lesion is not present about the cervix or in the folds of the vaginal mucosa. I do not know of any investigations of the vaginal and cervical secretions of such women for *Spirochaeta pallida* by rabbit inoculation tests, but such studies are advisable for shedding light upon this question. My belief is that *Sp. pallida* does not occur in the genital secretions of the woman unless an open lesion is present, although it is known that she may excrete *Sp. pallida* in the secretions of the mammary glands.

Experience teaches, therefore, that *Sp. pallida* is not ordinarily transmitted except by contact with open lesions or secretions of these. Such lesions are usually chancres and the cutaneous and mucous membrane lesions of the clinical secondary stage of syphilis. Tertiary lesions and their secretions are likewise infectious, but since they usually contain but few organisms, the infectiousness is only slight and may be altogether absent. The semen of syphilitic men may be infectious at any time after the primary stage, an instance of infection of a woman by the deposition of semen upon the labia being recently recorded by Moore and Keidel,[16a] but the ordinary body secretions as the saliva, perspiration and genital secretions of both sexes are probably not infectious in the absence of open lesions. Engman and Eberson[37] examined the nasal secretions of 24 syphilitics with negative results. *Spirochæta pallida* may be excreted in the urine, however, of either sex as indicated by the investigations of Vorpahl,[18] Hoffman,[19] Lévy and Guiél,[20] Fiessinger and Huber[21] and definitely proven by Warthin.[22] The latter found that this spirocheturia is most likely to occur during the spirochetemic stages of syphilis and is usually associated with degenerative lesions of the epithelium of the convoluted tubules rendering them more pervious to the passage of the organisms. It is possible that in women the urine may be a source of infection during sexual contact, but I do not know of any recorded instances of infection ascribed to this source. During the active stages of syphilis the mammary secretion of women may likewise contain *Sp. pallida*, indicating that when spirochetes are present in the blood they may find their way into the urine and to a lesser extent the milk, and possibly prove sources of infection in the absence of demonstrable open lesions.

It is commonly stated and quite generally accepted that *Spirochæta pallida* cannot produce infection of the skin or a mucous membrane unless the barrier of epithelium is first broken. Experiments with rabbits and monkeys and autoinoculation of human subjects consisting of rubbing *Sp. pallida* into the skin have commonly failed to produce infection. However, application of *Sp. pallida* to the vaginal mucosa of a rabbit presumably unbroken, yielded Reasoner[23] a positive result and Brown and Pearce[24] have succeeded in infecting the eyes of several rabbits by dropping *Sp. pallida* into presumably normal conjunctival sacs. Balzer and Barthelemy[25] have expressed the opinion that *Sp. pallida* deposited on normal integument, may under favorable conditions of warmth and moisture, proliferate, and penetrate the epidermis. Of course it is impossible to exclude the possibility and probability of minute abrasions of the skin and mucous membranes preceding infection with syphilis and for this reason it may never be possible to settle the question in a decisive manner whether or not pallida may gain access to the inter-epithelial lymph spaces through an absolutely intact layer of epithelial cells, but personally I believe this may occur on thin mucous membranes and especially if virulent *Sp. pallida* gain access to the glands.

From the standpoint of chemotherapy, this brief review of the mechanism of infection in acquired syphilis is of interest in relation to the prevention of

syphilis by the local destruction of *Sp. pallida*. This subject will be considered in more detail in Chapter LXX, but here it may be stated that the application of ointments of mercury or even thorough cleaning after exposure with soap and water may effectually kill *Sp. pallida* before deep invasion has occurred. This system of prophylaxis has been especially applied to men with a considerable degree of success providing the prophylactic measures are applied within an hour after exposure. Women are less frequently protected, because of the greater difficulty of applying the prophylactic procedures. The same measures may be applied when extragenital infection is feared. I have known two men, escaping a genital infection after contact with syphilitic women presumably due to the application of calomel ointment, to contract syphilis of the lips, and the application of prophylactic measure should not be neglected when extragenital infection may occur.

When more than three or four hours have elapsed after exposure reliance upon local prophylaxis in either sex cannot be placed upon local measures alone; under these circumstances arsphenamin or neoarsphenamin should be administered, as likewise to women who fear seminal infection after contact with Wassermann positive men.

Mechanism of Primary Infection in Maternal and Congenital Syphilis.—Syphilis of the woman presents certain peculiarities as yet little understood. In a broad and general manner the disease is not ordinarily as severe in her as in the male. The chancre may be and frequently is, so small and inconspicuous as to escape detection; the adenopathy and cutaneous lesions may be just as severe, but she is more likely to escape tertiary lesions of the nervous and cardiovascular systems. Probably this is due to the fact that heretofore she has not been subjected in the same degree as men to the wear and tear of these organs, which doubtless favors the localization and activities of *Sp. pallida* in these tissues. The latent periods in the tertiary stage are usually quite prolonged and in not a few instances the only symptoms for years and years are positive Wassermann reactions and the birth of frankly syphilitic children or children below par in general health. Even when there are reasons for strongly suspecting syphilis as a result of giving birth to syphilitic offspring, the Wassermann reaction is frequently negative with the total absence of lesions and symptoms indicating that some mechanism is operative in holding in check the activities and virulence of pallida in her tissues. Doubtless immunological processes are operative in women as a result of pregnancy which are not developed in men and childless women, but these will be discussed in more detail in Chapter XXXVII.

Undoubtedly women are infected with *Sp. pallida* in the semen of syphilitic men and especially during the first four years of the disease. It is well known that the testicles are commonly involved after the primary stage. In both man and the lower animals *Sp. pallida* frequently locate in these tissues although my colleague Dr. Saleeby, has found almost no evidence at all of syphilitic infection of the seminal vesicles of some twenty odd cases of tertiary syphilis, removed at necropsies and examined histologically. Neisser[26] states that in his experiments with apes "it appeared that the testicles acted particularly well and long as depots of the virus." Nichols[27] observed that after the intravenous injection of *Sp. pallida* in rabbits, the testicles were frequently the only organs to develop lesions. Symmers[28] observed chronic interstitial orchitis in 67 or 39 per cent. of a series of necropsies upon 171 male subjects with late acquired syphilis and Warthin[29] was frequently able to demonstrate *Sp. pallida* in testicles presenting no gross lesions. As stated by Herman and Klauder[30] clinical syphilis of the testicles is rare, that is, the presence of demonstrable lesion and particularly the typical "billiard

ball" lesion of chronic fibrosis is seldom encountered but nevertheless, histological examination of the testicles of syphilitic subjects shows that they are more frequently involved than indicated clinically. Syphilis of the prostate gland on the other hand, is quite rare as indicated by a recent analysis of the literature by Thompson,[31] and syphilis of the seminal vesicles has not yet been demonstrated.

Despite the frequency with which the testicles may be involved, the semen from syphilitic men frequently is not infectious for rabbits when injected into the testicles. Finger and Landsteiner[32] in a series of four men found *Sp. pallida* in the semen of two cases, one with orchitis and a second without demonstrable involvement of the testicles. Uhlenbuth and Mulzer[33] and Neisser and Hoffmann[34] have recorded additional positive findings in the semen during the active stages of syphilis and Engman and Eberson[35] found active, virulent *Sp. pallida* in the semen of two of a series of seventeen subjects with latent syphilis. Klauder[36] inoculated rabbits intratesticularly with 1 to 2 c.c. of semen from each of twenty-two subjects (one in primary stage, eight in secondary stage and thirteen in latent and tertiary stages) with negative results in all. Of course these examinations were usually made with one specimen from each subject, whereas it is easily possible and indeed probable, that the semen may be infectious at one time and not at another.

At any rate, it is well known that women may be infected with the semen of syphilitic men presenting no demonstrable lesions or symptoms of syphilis and even regarded as "cured." I do not know of a single instance, however, of a primary lesion being found in these cases. It is generally believed that the women is infected in a secondary manner; that is, *Sp. pallida* infects her ovum and that she in turn is gradually infected by the syphilized product of conception. I believe it is possible, however, for the spirochete to infect her directly without impregnation and without the production of a demonstrable primary lesion, the parasites gaining access to her lymphatic system by way of the endometrium. The resulting disease, however, is generally latent and may present nothing but a positive Wassermann reaction for many years. The causes and nature of this latency are, however, unknown. Possibly the parasites have been rendered comparatively non-virulent by residence in the tissues of the male or by reason of some profound alteration in their life cycle. Probably a syphilitic fetus aids in the development of immune principles in the woman counteracting the proliferative activities, virulence and dissemination of *Sp. pallida*, but there is no direct evidence of such. Possibly the secretions of the endocrine glands of the woman exert a restraining influence upon *Sp. pallida* as suggested by Montgomery,[37] who states that iodothyrin of the thyroid gland may be responsible since disturbances and activity of this gland is comparatively common among women.

The mechanism of prenatal or congenital syphilis is frequently a puzzling phenomenon. Of course a woman contracting syphilis after conception may transmit the spirochetes to the fetus any time up to the seventh or eighth months, the organisms passing from the maternal to the fetal blood. After the eighth month the child commonly escapes by being born before the spirochetemic stage of the disease has been well-developed in the mother. Likewise a syphilitic woman is always apt to transmit *Sp. pallida* to her child in the uterus for many years, the parasites being transmitted to the fetus by way of the blood in just the same manner as they may progressively invade any of her own tissues and organs. If the infection is latent in the mother, the child may escape infection but anything that stirs up the infection in the mother increasing the multiplication and virulence of the spirochetes

with invasion of the lymph and finally of the blood, increases the danger and likelihood of infection of the child. A child may likewise contract syphilis during birth if the mother happens to have an open lesion about the genital organs at this time.

But is there a strictly paternal form of transmission in prenatal syphilis? In other words, may the father directly infect the ovum at the time of its conception? Clinically this is believed by many to be possible and that the child may be infected and the mother escape. From the biological standpoint, however, paternal infection of the ovum is doubtful and at any event, infection of a child without subsequent infection of the mother is very improbable.

The head of a spermatozoön ordinarily measures 5 microns in length, 4 in breadth and 2 in thickness; *Spirochæta pallida* measures from 6 to 14 microns in length and from 0.2 to 0.5 in thickness. It is possible, therefore, that the head of a spermatozoön may mechanically carry a spirochete into an ovum which approximately measures 5 microns in size, but this does not seem to be probable. However, it is possible as previously discussed, that a granular stage of *Spirochæta pallida* may exist and if such is true, direct infection of the ovum may be readily understood.

It is my opinion that if an infectious granular or spore stage of *Sp. pallida* does not exist, that in paternal transmission of syphilis, *Spirochæta pallida* in the semen is taken directly into the uterine canal followed by infection of the pelvic lymphatics of the mother with secondary infection of the fetus. The end result is the same as if the spirochete invaded the ovum direct with a spermatozoön, only if this hypothesis is correct, the woman is always infected primarily and the fetus secondarily rather than the fetus primarily and the mother secondarily.

The subject of maternal and fetal syphilitic infection is of great interest and importance in relation to chemotherapy and will be discussed in more detail in Chapter LXXVII. Here it may be stated that a syphilitic father does not always transmit the disease. An abundance of clinical evidence shows that during the latent and tertiary stages he may beget apparently non-syphilitic children and we know that the semen may not contain *Sp. pallida* infections at least for the testicles of rabbits. But, however, it is also known that in some cases the testicles of the father may be invaded with *Sp. pallida* even though they appear clinically normal and from this standpoint the uncured male is potentially liable to transmit the disease in his semen and is usually a fit subject for treatment.

For the same reason, the mother of a child to a syphilitic man is apt to be infected even though she is apparently free of symptoms and lesions. As previously stated, the woman under these circumstances presents the most striking degrees of latency, the mechanism of which has not been satisfactorily explained. For this reason I believe that such women likewise demand treatment and especially if the child shows any signs or symptoms whatsoever suggestive of syphilis. However, a uniform rule to treat all women bearing children to syphilitic fathers would probably include some women who are not syphilitic; this is especially true if many years (more than five) have elapsed since the father's primary infection and if he has had some treatment and if the infection was clinically latent (although the Wassermann reaction may be positive) at the time of conception.

Theoretically every child of a syphilitic father or a syphilitic mother or both, should be treated for syphilis at least for a brief time, even though apparently free of lesions and symptoms. Children born without manifest lesions may still be infected and in such cases the latency is analogous to

latency in the mother. Our knowledge of the clinical symptoms and lesions of latent congenital syphilis is being constantly increased and we now know that in children certain lesions and symptoms are syphilitic which a few years ago were not regarded as such. However, a uniform rule to treat all children for syphilis born of a father who may show nothing more than a positive Wassermann reaction would be practically sure to include some who were not actually infected. The same is true but to a much lesser extent, of children born of a woman who presented nothing but a positive Wassermann reaction, but the chances of a child escaping under these conditions is so slight that it should be the rule to treat every such child regardless of the fact that clinically there are apparently no signs of infection.

Mechanism of Systemic Infection in Syphilis.—Within forty-eight hours after exposure and inoculation the spirochetes of syphilis have probably gained access to the lymph spaces in the deeper layers of the skin or mucous membrane and it is likely that a few parasites have already invaded the nearest lymph glands; invasion of the glands is surely the case in the majority of infections after the primary lesion has developed.

The spirochetes apparently proliferate rapidly in the lymph and lymphatic glands and being swept into the venous blood are carried to the heart, the lungs and arterial blood followed by distribution through the coronaries and other arteries. Subsequently they are lodged in the arterioles and invade the perivascular spaces, setting up there a train of pathological changes consisting mainly of chronic hyperemia, lymphocytic, plasma cell and serous infiltration accompanied by necrosis of the cells of the tissue invaded and usually ending in fibrosis of the lesions of long duration.

From these depots of infection the spirochetes may progressively involve an organ by direct extension along interstitial and especially lymphatic spaces, yielding uniformly distributed and fibrotic lesions. From these localized or general lesions, organisms are likely at any time to be swept along with the lymph into the venous blood followed by arterial distribution to parts of the same or distant organs with localization by preference in tissues or organs the seat of previous injury or active hyperemia, including the placenta and fetus in the case of pregnancy. From the standpoint of this localization of spirochetes from the primary lesion or from these secondary depots, the subjects of special strains and selective localization are of much interest and importance, which I shall discuss in the succeeding chapter.

Spirochetemia or the presence of spirochetes in the blood, is, of course, most marked during the few weeks following the development of the primary lesion and before the development of the cutaneous and mucous membrane lesions of the clinical so-called secondary stage of the disease, but subsequent to this and at any time thereafter, temporary blood infections may occur and especially if active lesions are present anywhere in the body. Since clinically latent lesions may be, therefore, foci from which spirochetes are distributed to other organs and tissues, it would appear that the syphilitic is always a fit subject for treatment unless this is contraindicated by senility, by pathological changes in vital organs reducing tolerance for spirocheticidal compounds or by syphilitic lesions so situated or developed and especially in the central nervous system, that therapeutic success cannot be expected and it is decided to leave well enough alone. From the standpoint of chemotherapy best results are secured of course, before lesions become fibrotic, this subject being discussed in more detail in Chapter XXXVII dealing with the pathology of syphilis in relation to chemotherapy.

Relation of Trauma and Tissue Injury to Syphilitic Infection.—Trauma in a broad sense probably bears an important relation to the dissemination and localization of *Spirochæta pallida*. I have already mentioned that trauma greatly predisposes to initial infection and subsequent to this time, trauma or tissue injury in a broad sense and caused by many different agents in an important factor in the future welfare of the infected individual.

Physical trauma as long continued irritation at one point, fractures, surgical operations, etc., may apparently favor the development of *Spirochæta pallida* in these areas or if the tissue injury involves a focus of syphilitic infection, the spirochetes may be stimulated into reproductive activity and virulence. Klauder[38] has recorded cases of gummatous ulceration developing at the site of lacerations, after repeated and long continued trauma and after injury to the testicles; also of provocative Wassermann reactions following a surgical operation and the injection of antitoxin, both procedures apparently stimulating latent foci of spirochetes into activity. Gougerot and Clara,[39] Ramond and Postina[40] and Coues[41] have recorded instances of lesions developing in the skin, muscles and bones at the site of wounds and contusions inflicted during warfare. Estes,[42] Gelle',[43], Charpy[44] and others have reported instances of non union of fractures due to syphilis and presumably the presence or localization of *Sp. pallida* in the periosteum and marrow. Klauder states that there is no doubt that trauma can be ascribed as a precipitating cause of paresis and in an analysis of sixty one cases among admissions to the Boston Psychopathic Hospital, symptoms of paresis followed recent trauma in three cases. In other cases physical exhaustion, sunstroke and lead poisoning appeared to initiate the symptoms of paresis. Osnato[45] has also reported cases of post-traumatic paresis developing within a few days to a few weeks after injury and Pighini[46] states that during the late war violet emotions and injuries of the skull and the dorsal column may have been the indirect cause of the appearance of some of the serious disorders among Italian soldiers, primarily due to syphilis. Southard and Solomon[47] state that on general grounds post-traumatic effects are not likely to be delayed more than three months after injury.

It is not difficult to understand that if an injury involves syphilitic tissue, the spirochetes may spring into renewed activity since the conditions for multiplication would be favored in the devitalized tissue and inflammatory exudates. Likewise, if injury should occur during or before spirochetemia, it is highly probable that localization in such areas would be favored by reason of hyperemia and cultural conditions. In a general way, any profound local or systemic injury would exert effects of this kind favorable to syphilitic infection and among general or systemic injuries I would include the effects of pregnancy, chronic alcoholism and other intoxications, excessive mental work, worry and exhaustion, etc., as factors reducing local or general resistance of an isolated organ or the body as a whole to the dissemination of *Sp. pallida*, the arousing of latent foci and the progression of syphilis.

In view of the widespread prevalence of syphilis it cannot be said that the development of syphilitic lesions at the sites of injuries is a frequent occurrence; this is readily understood when we keep in mind the intermittent character of the spirochetemia in syphilis and that traumatic injuries may only occasionally actually involve nests of *Sp. pallida*. Klauder, however, has thoroughly discussed trauma in relation to syphilis from the medico-legal standpoint, in which relation the subject is of considerable importance.

From the standpoint of the treatment of syphilis, the subject is likewise of considerable importance and especially in relation to tardiness in the healing of wounds and the non-union of fractures as well as again emphasizing the advisabiiity of treating latent cases of syphilis unless distinctly contra-indicated, if for no other reason than the danger that constantly exists of local or general tissue injury stimulating latent foci into renewed activity.

Pathogenesis or Mechanism of Production of Disease by Spirochæta Pallida.—By the pathogenesis of *Spirochæta pallida* I refer to the agencies by which these spirochetes actually produce the lesions of syphilis—that is, the mechanism of the production of the disease syphilis. We know that bac-

teria may produce disease by a wide variety of agencies as true exogenous toxins, endotoxins and aggressins, ferments, bacterial proteins, the products of necrosis of inflammatory exudates, etc. By what agencies are syphilitic lesions produced?

There is surprisingly little data on this subject. Clinically it is supposed that *Sp. pallida* produces a toxin or toxins to explain the anemia, loss of weight, general body pains and such general symptoms of infection in syphilis but no one has yet clearly demonstrated the existence and nature of these substances. From the pathological standpoint it appears almost certain that virulent *Sp. pallida* produce toxic substances in the tissues responsible for the necrosis of the cells of the tissue infected and for exerting a positive chemotaxis on small lymphocytes.

It is also possible that endotoxins in the nature of aggressins are produced, since phagocytosis of *Sp. pallida* is so rarely seen in sections of syphilitic tissue or smears of inflammatory exudates. Proteolytic ferments, however, apparently are not produced since caseation and liquefaction necrosis do not occur except in large and old lesions in which case vascular occlusion and loss of nutrition are primarily responsible. Syphilitic lesions in the skin and mucous membranes open to secondary bacterial infection frequently suppurate, but these effects are ascribed to the toxins and ferments of the bacteria rather than to spirochetic agents. In malignant syphilis, however, the large size and necrotic character of the lesions with profound symptoms of toxinemia, may be ascribed primarily to toxic substances elaborated by unusually virulent pallida.

From the standpoint of treatment it may be stated that all attempts have failed toward developing a successful method of serum therapy for the destruction of the spirochetes and the neutralization of their toxins. Some investigators claim that upon the rapid destruction of large numbers of *Sp. pallida* by such spirocheticidal agents as arsphenamin and neoarsphenamin, that poisonous products are released which may be responsible in part for the symptoms and lesions constituting the reaction sometimes following the administration of these compounds. Indeed, this is one of the oldest explanations of the mechanism of the arsphenamin reaction and in part it may be true in some cases at least, but I shall discuss this subject more fully in Chapter XLVII.

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CHAPTER XXXVI

STRAINS OF SPIROCHÆTA PALLIDA IN RELATION TO THE TREATMENT OF SYPHILIS

It is of course well known that the clinical manifestations of syphilis are subject to wide variations in all stages of the disease. Even the primary lesion may vary from a slight superficial erosion which may escape detection, to a large phagedemic ulcer and the cutaneous and mucous membrane lesions may vary from so few in size and number as to almost escape detection to those that are large, numerous and even pustular. The late lesions, however, are the most variable in number and location. In some cases they are principally located in the tissues of the central nervous system producing paresis or tabes dorsalis as the predominating and principal lesions; in other cases these tissues seemingly escape while the myocardium, aorta, the skeletal system or other organs are heavily infected.

What are the determining factors? Do special strains of *Spirochæta pallida* exist with inherent transmissible selective affinities for the brain and nerve tissue in one case and for the cardiovascular or cutaneous organs in another? The subject is one of importance and especially so in relation to treatment and prognosis since there is considerable variation in the curability of these lesions, those of the brain and spinal cord being much more dangerous

and less amenable to treatment than those of the cutaneous and skeletal tissues.

Variations in the Virulence of Spirochæta Pallida.—In the first place, I believe it is to be granted that *Spirochæta pallida* varies considerably in virulence, by which is meant variations in the degree of invasiveness of the tissues, rate of multiplication and the production of those offensive agencies designated as toxins. It is known that this occurs in experimental infections of rabbits and that inoculation with spirochetes from active syphilitic lesions are followed by more rapid development of scrotal and testicular lesions than follows inoculation with spirochetes from old and relatively inactive lesions. Among the bacteria it is well known that variations in virulence occur and that the resulting diseases vary in clinical severity and duration accordingly without involving the question of specific strains; I can see no reason why the same may not be true of the pathogenic spirochetes including *Spirochæta pallida*. From the clinical standpoint it is commonly observed that inoculations with spirochetes from acute chancres and mucous patches results in the production of severer primary lesions than inoculation with organisms from older and latent lesions; in this connection it has been suggested that the virulence of *Spirochæta pallida* in the skin and bones may be reduced by the action of ferments and consequently rendered less invasive for the central nervous system. Malignant syphilis may not be necessarily due to infection with a particularly virulent strain of *Spirochæta pallida*, but rather a greatly reduced resistance of the victim to infection. The severity of all cases of syphilis among human beings and those produced experimentally in the lower animals, are doubtless influenced by the state of natural resistance of the host and the degree of defensive activity, but in addition we doubtless have variations in the virulence of the parasites themselves to account for the varying degrees of severity of the resulting lesions.

I believe, therefore, that inherent virulence of the strain of *Spirochæta pallida* producing the infection, together with the degree of local and general resistance offered by the host and more especially, the location of areas of reduced resistance in the host the result of trauma, previous disease or even of inheritance, largely determines the distribution and localization of spirochetes and consequently the clinical course of syphilis in any given case without involving the question of specific organotropic strains. However, a large amount of experimental work has been published lending support to the theory that specific strains of *Spirochæta pallida* exist and while these are not conclusive, they are worthy of the closest attention in view of the great importance of the subject.

Influence of Local Reactions upon the Distribution of Spirochæta Pallida. When *Spirochæta pallida* are deposited in the tissues either at the time of primary infection by direct inoculation through abrasions of the epithelial barriers or subsequently by the lymph and blood, the tissues of the host react to the invasions with the production of local reactions consisting of the usual cycle of inflammatory phenomena embracing hyperemia with serous and cellular exudation. This occurs with primary inoculation and the reactionary lesion is called a chancre; it occurs in the spirochetemic secondary stage and the lesions are called papular syphilids, mucous patches, etc. Exactly the same kinds of lesions occur in the internal organs hidden from view.

Each and every local reaction expresses the defense of the host to this parasitic invasion; it is Nature's means for resisting the infection. We know little or nothing of the exact nature and mechanism of the defensive forces at work in each of these foci, but investigations have shown that they apparently influence the multiplication, subsequent distribution and possibly the

pathogenic properties of the spirochetes. Evidently the power of tissue reaction varies in different organs during the early stages of the disease; in other words, during the spirochetemic stage of syphilis prior to the development of the secondary lesions, the parasites are doubtless distributed broadcast throughout the body, but in some tissues they apparently either do not find conditions favorable for survival or they are immediately destroyed by defensive agencies. Later in the disease these tissues may be invaded again and again with finally the successful establishment of foci of infection, particularly in areas of reduced resistance; but in this case, invasion is by spirochetes with acquired resistance to these defensive agencies and thereby capable of surviving and establishing infection. At any rate, even in paresis the other internal organs do not escape; syphilitic aortitis and myocarditis especially may be present as well as cutaneous and osseous lesions showing that the spirochetes are not specific for the nervous tissue alone, granting that specific tissue strains actually exist.

Nichols[1] has shown that removal of the local focus in one testicle of a rabbit favors development of syphilis in other and since then Brown and Pearce[2] have shown in a clear and decisive manner, that suppression or removal of a local testicular lesion in a rabbit favors the widespread distribution of the spirochetes in this animal. In other words, it would appear that in the local reaction to *Spirochæta pallida* in the initial chancre, the body cells of the host produce something which is not only inimical to the spirochetes in the lesion but which apparently holds in check the multiplication, distribution and pathogenic properties of those spirochetes already located in the neighboring lymphatic glands and other tissues.

Now clinical experience confirms the teaching of Fournier that human subjects developing large chancres followed by florid secondary lesions are less likely to develop infection of the nervous system and other internal organs and are more readily cured than subjects developing small chancres and poorly developed secondaries. In other words, clinical experience is in conformity with experimental work, showing that when the host is able to produce a large local reaction about invading spirochetes something is produced that tends to destroy or at least to hold in check the multiplication, virulence and distribution of the parasites.

Just what determines the degree of this local reaction is unknown. Neither do we know of any measures by which it may be increased for therapeutic purposes. It is apparently controlled by inherent forces of natural immunity and resistance, concerning which we know practically nothing at all insofar as syphilis is concerned. Apparently it bears no relation to general health; the strong and robust may have the small chancre and early neural infection while the frail and sickly may have the large chancre and delayed tertiary lesions with clinical escape of brain infection.

According to Marie and Levaditi the so-called neurotropic strains of *Spirochæta pallida* produce the small chancres in human beings and rabbits; it may be that the degree of local reaction is dependent upon the properties of the spirochetes and not upon the defensive agencies of the host. If such were true the degree and kind of local reaction would be evidence in favor of the existence of specific strains of *Spirochæta pallida;* but be this as it may, it would appear that the character of the primary local reaction (chancre) and the subsequent cutaneous and mucous membrane reactions (the secondaries) appear to influence the internal distribution and virulence of *Spirochæta pallida*, even though the character of these lesions may in itself be evidence of inherent tissue specificity on the part of different strains of pallida.

Trauma and Tissue Injury in Relation to the Distribution and Specific Strains of Spirochæta Pallida.—As discussed in the preceding chapter, trauma and tissue injury in a broad and general manner exerts an important influence upon the development of the late lesions of syphilis. It is readily understood how and why this is the case when the tissue injury involves a latent focus of *Spirochæta pallida* infection, the multiplication and even the virulence of the spirochetes being enhanced by a lowering of local resistance. But does tissue injury influence the localization of the parasites during the spirochetemic stages of syphilis?

In this sense I believe that the subject of tissue injury must be broadly interpreted. For example, the laborer engaged in heavy work with temporary, but frequently repeated, high blood pressures may injure his aorta or myocardium and favor localization of spirochetes in these organs. The individual engaged in severe and prolonged mental work, strain or worry may injure the tissues of the brain and favor localization of the parasites in this organ with the eventual production of paresis or some other syphilitic disease of these tissues, even though it may not be possible to evaluate the degree of predisposing tissue injury by morphological changes. Possibly even long continued eye strain due to errors of refraction may so influence vascular changes in the choroid and retina as to favor the localization of spirochetes in these tissues.

I believe that tissue injuries of this sort producing local areas of lowered resistance or long continued congestion influence the localization of the spirochetes not only during the primary period of spirchetemia following the chancre and preceding the cutaneous eruption, but also throughout the existence of uncured syphilis. As previously stated it is highly probable that temporary spirochetemias are of frequent occurrence even in the late stages of syphilis and trauma or tissue injury may continue to exert an influence upon localization of spirochetes throughout the course of the disease. In this manner we may explain the frequency of syphilitic mucous patches and glossitis in smokers; the squamous syphilids of the palms among those engaged in manual labor and the lesions developing after tatooing etc.; Chesney and Kemp[3] have recently shown in experimental syphilis of rabbits that *Spirochæta pallida* can be inoculated into granulating ulcers of the skin and that active proliferation of connective and epithelial cells greatly favors the multiplication of *Spirochæta pallida.*

Hereditary Influences in Relation to the Specific Strains of Spirochæta Pallida.—An additional factor of importance in relation to this question of specific strains of *Spirochæta pallida* is that of familial predisposition to parenchymatous neurosyphilis with special reference to paresis and tabes dorsalis. Older syphilologists frequently mentioned the tendency for these forms of syphilis to develop in members of the same family.

Recently Moore and Keidel[4] reported three families embracing eight members, all of whom acquired syphilis at different times and from different sources, yet all developed neurosyphilis. Moore and Kemp[5] subsequently studied two additional families. In the first, two brothers, acquiring syphilis from different women, at widely separated dates, developed general paresis. In the second family, the father acquired syphilis in 1900 and died in 1918 of paresis; his son contracted the disease in 1919, and in 1923 developed typical taboparesis. As stated by Moore and Kemp, the fact that in five families such an occurrence has been observed is too striking for mere coincidence; it would appear that in certain families, the nervous system is a joint of local reduction in resistance to *Spirochæta pallida.* Granting that such predisposition of the brain and cord to infection were transmissible, mere invasion of these tissues with *Spirochæta pallida* during the early or initial spirochetemic stage of syphilis would be sufficient to bring about localization without involving the question of specific neurotopism. Kemp and Poole[6] have recently stated however, that on the basis of studies on the incidence of neurospyhilis among the parents of congenitally

neurosyphilitic children in twenty familes, it was found that neurosyphilis was eight times as frequent among the mothers and over three times as frequent among the fathers of congenitally neurosyphilitic children as among the parents of children with congenital syphilis without demonstrable neurosyphilis. In their opinion this difference is sufficiently striking to suggest the existence of a neurotropic strain of *Spirochæta pallida* responsible for the production of neurosyphilis although familial predisposition to neurosyphilis may be a factor of equal importance in determining this type of the disease. Indeed as stated by Cooke and Jeans[6a] recorded instances of twins born to syphilitics, one of whom developed neurosyphilis and the other not, apparently constitutes evidence against the existence of a neurotropic strain of *Spirochæta pallida*.

Clinical Evidence Concerning Strains of Spirochæta Pallida.—Probably the strongest evidence in favor of specific tissue strains of *Spirochæta pallida* is derived from clinical studies of these kinds. Unfortunately, however, clinical studies and deductions are subject to much error and require great caution before adoption. Mention has already been made of the clinical relation between the small chancre, and the poorly developed or absent cutaneous lesions with the early development of visceral syphilis, including paresis and tabes. But these events may be explained just as well on the basis of the virulence (invasiveness) of *Spirochæta pallida*, the character of the local defensive reactions and the predisposing tissue injuries as upon the supposition that such infections are due to specific strains of *Spirochæta pallida*.

The strongest clinical evidence of neurotropic strains of *Spirochæta pallida* is based upon the few historical instances of doubtful authenticity, purporting to show the infection of different men with paretic strains of *Spirochæta pallida* from the same women; also the incidence of conjugal neurosyphilis which is the development of neurosyphilis among the wives of neurosyphilitic males or of the husbands of neurosyphilitic females.

Fischer[7] found only ten cases of conjugal neurosyphilis among the partners of 395 paretics but Meyer[8] and Hauptman[9] observed that about 30 per cent. of partners developed neurosyphilis. More complete studies by Plaut and Goring[10] have shown that 60 per cent. of the partners of neurosyphilitics are syphilitic; that 18 per cent. of the total number of partners are neurosyphilitic and that about 30 per cent. of the syphilitic partners are neurosyphilitic. These results, however, were based almost solely upon symptomatic evidences alone; studies including the routine examination of the spinal fluid for evidences of asymptomatic neurosyphilis have shown a higher percentage of neural involvements as indicated by the reports of Wile and Marshall,[11] Moore and Keidel[12] and Moore and Kemp.[13] The data offered by these investigators suggest the existence of a neurotropic strain of *Spirochæta pallida* as an etiologic factor in paresis and tabes, but not in diffuse cerebrospinal syphilis, in which cases evidence supporting the theory of special strain is lacking. While about 75 per cent. of the partners of neurosyphilitics develop syphilis, although in the majority of these the disease is latent and detected only by serological and other examinations of the blood and spinal fluid, in only a small number does the conjugal infection run true to the type of the partner; furthermore, it is entirely likely that if it were possible to examine more closely the other internal organs and especially the heart, aorta, kidneys, adrenals and testicles it would be found that the spirochetes were more generally distributed than alone to the brain, cord and meninges.

Sicard[13] has refused to accept the evidence indicating the existence of specific tissue strains of *Spirochæta pallida* and states that neurotropic strain is not likely to be transmitted from one person to another except during the chancre stage, inasmuch as the cutaneous and mucous membrane lesions are regarded as of uncommon occurrence. If this is true Sicard claims that neurotropic strains are destined to disappear, because the opportunities for

contagion are rare; but as a matter of fact syphilis of the nervous system is apparently increasing instead of decreasing. However, Sicard overlooks the fact that a syphilitic man may possibly transmit a neurotropic strain in his semen since temporary spirochetemias occur in neurosyphilis just as in other clinical types with involvement of the testicles. It is likewise true that a neurosyphilitic woman may develop open lesions with the infection of her sexual partner, or of her children by direct placental transmission of the strain during temporary periods of spirochetemia. While the danger of infection for the spouse increases as the date of infection in the one acquiring syphilis approaches the time of marriage, yet it is well known that neurosyphilitic patients may be infectious for many years, in spite of the absence of demonstrable open lesions. From the clinical standpoint, therefore, the theory of the existence of neurotropic strains of *Spirochæta pallida* has some support but the evidence is not conclusive.

Experimental Syphilis of the Rabbit in Relation to Specific Strains of Spirochæta Pallida.—The experimental work upon which the theory of specific tissue strains of *Spirochæta pallida* is based, was first conducted in this country. From the standpoint of morphological differences in strains Noguchi[14] first described thin, thick and average types and what is more important in relation to the possibility of specific strains, that the thin type usually produces rapidly growing and diffuse lesions. Since then it has been shown, however, that these morphological types do not bear any constant relation to the generalized lesions of syphilis in the rabbit or human being, Reasoner,[15] for example, reports that no morphological differences were discernible among those strains producing choroiditis and chorioretinitis of rabbits and those that did not. Pfeiffer[16] has reported that both thick and thin forms have been observed among spirochetes recovered by him from four cases of human paresis and Levaditi and Marie have not noted any constant morphological characteristics of their so-called dermotropic and neurotropic strains.

In 1913 Nichols and Hough[17] secured a strain of *Spirochæta pallida* from the spinal fluid of a case of nervous relapse following the administration of salvarsan in the secondary stage of the disease. The parasite was of the thick variety, produced hard, nodular lesions in the testicles of rabbits and was characterized by high invasive power with a tendency to produce generalized lesions, and especially of the skin and eye. Subsequently Nichols[18] expressed his belief that while all strains probably localize indifferently in certain organs during the early septicemic stage, in addition, certain strains probably have an especial localizing power.

Reasoner subsequently reviewed this work, reporting upon the characteristics of this and additional strains, two of which likewise produced choroiditis and chorioretinitis. Fourteen other strains from chancres and mucous patches did not produce these lesions. From a large number of experiments on rabbits he concluded that it is possible to demonstrate satisfactorily fixed differences in various strains of *Spirochæta pallida* as found in the lesions produced in rabbits and that it is possible to produce involvement of nervous tissue without actual intracranial inoculation. He was careful enough to add, however, that in evaluating these results in relation to human syphilis, individual resistance of the infected person must still be considered as a factor in the localization and development of the disease.

Since then the strain of *Spirochæta pallida* isolated by Nichols and Hough has been widely employed in this country for experimental work with rabbits. It still maintains a high degree of invasiveness and as stated in Chapter XXI, Brown and Pearce have employed it in much of their work with the production of widespread generalized lesions, especially after suppression of the primary lesion. I have likewise employed it in my work for the past eight years with essentially similar results. It cannot be said, however, to possess any type specificity other than a high degree of invasiveness. Zinsser and his colleagues have compared its pathogenicity along with five other strains isolated from mucous patches and chancres and found the lesions essentially similar for all.

In Europe the investigation of Levaditi and Marie[19] upon specific types of *Spirochæta pallida* have attracted a great deal of attention. Both are strongly of the opinion that

there are two independent strains, one of which they term the *dermotropic* and the other the *neurotropic*, in accordance with their selective localization. They have suggested that the neurotropic strain may be derived by evolution from a common parent by propagation in the nervous tissue giving rise to a really new strain that may persist like a mendelian variant, thereafter functioning as such instead of reverting to its former characteristics.

They claim that the dermotropic and neurotropic strains may be differentiated according to the lesions produced in rabbits, the neurotropic strains having a longer period of incubation and producing superficial and erosive papulosquamous lesions in contrast to the deep, ulcering lesions with hard bases produced by the dermotropic strains. The neurotropic strains according to these investigations attack epithelium with a special affinity for the prickle cells while the dermotropic strains produce vascular and sclerotic changes. The latter have been found pathogenic for the lower monkeys, anthropoid apes and human beings, while the neurotropic strains were found non-pathogenic for these when inoculated cutaneously.

What has been most striking to me has been their reports indicating that rabbits infected with the dermotropic strains are not immune to superinfection with neurotropic strains and the reverse. Among human beings there is almost an absolute immunity to re-inoculation with *Spirochæta pallida* after the lesions of syphilis are well developed, but according to Levaditi and Marie, a human being infected with a neurotropic strain would not be immune to a dermotropic strain and the reverse. Among those who regard *Spirochæta pertenuis* of yaws a distinct and different organism from *Spirochæta pallida*, great emphasis is placed upon the fact that a monkey or rabbit infected with yaws is susceptible to syphilis and the reverse, that human cases of yaws may contract syphilis and that syphilitics may contract yaws. If Levaditi and Marie are correct, however, the significance of these immunological differentiation is greatly reduced and weight is added to the view that *Spirochæta pertenuis* is really a dermotropic strain of *Spirochæta pallida.* Levaditi and Marie have mentioned in this connection that immunity to any one of the three different spirochetes of European, African and American relapsing fevers, does not protect from infection with the others, in spite of morphological resemblances and the similarity of the disease which they produce.

Fournier and Schwartz[20] have described two kinds of scrotal lesions in rabbits which appear to be constant. One type produces a herpetiform, erosive lesion after an incubation period of fourteen to twenty-one days, and lasting about three weeks, while the other produces a deep, indurated ulcer after an incubation of one to one and one-half months which persists for one to three months.

Pagniez[21] and numerous others have also expressed their belief in the existence of a neurotropic strain but since the discovery of *Spirochæta cuniculi*, which produces a spontaneous venereal disease of rabbits with superficial papulosquamous lesions strikingly similar to those produced by the neurotropic strains, one cannot escape the suspicion that these spirochetes may have been confused with *Spirochæta pallida;* however, in a recent paper by Levaditi and Marie[22] this possibility was considered and apparently excluded to their satisfaction. Laumonier,[23] Sicard,[24] Sezary,[25] Marchand,[26] Plaut and Mulzer[27] have refused to accept the evidence and theory of the plurality of strains of *Spirochæta pallida*, believing that a difference in the host rather than in the organism itself accounts for the variance in results.

More recently Wilkes-Weiss, Richter and myself[27a] have studied this problem in a different way, namely, by immunizing rabbits with six different cultures of *Spirochæta pallida* including the Nichols-Hough strain, and then studying the question of immunological specificity by means of cross agglutination and complement-fixation tests after the manner by which pneumococci and other organisms have been classified into groups. Our results however, have been of a negative character, that is to say, we were unable to demonstrate any evidences of immunological specificity on the part of the different strains employed by us.

But I believe that more work is required to settle this question and especially cross immunization tests as conducted by us to determine more accurately the relation or lack of relation between strains. It is especially necessary to be absolutely sure that *Spirochæta cuniculi* is not included.

Concerning agglutinin production, it appeared in my experiments that cultures of many different strains showed practically no variations in antigenic activity for rabbits. I believe that the experimental evidence in favor of accepting a specific neurotropic strain of *Spirochæta pallida* is as yet inconclusive. Strains of higher invasive power than the average may be isolated, as originally accomplished by Nichols and Hough, and for which they claimed this attribute only insofar as strain specificity was concerned. Further than this, however, I do not believe conclusions are warranted, since the distribution and localization of *Spirochæta pallida* are so greatly influenced in the experimental animal by the inherent invasive qualities of the spirochetes together with the nature and degree of the local primary reaction and the occurrence of trauma and tissue injury.

Immunological Phenomena in Syphilis in Relation to Localization of Spirochæta Pallida.—After infection with *Spirochæta pallida* has occurred do immunological processes determine or influence the subsequent localization of the parasites? We have already discussed the importance of tissue injury as a predisposing factor in localization but may tissues and organs escape infection by possessing a higher degree of resistance than the average? Or may the reverse occur with the production of allergic sensitization of some organs and notably the nervous tissues, as the result of long continued infection, with predisposition to the localization of *Spirochæta pallida* as suggested by Noguchi?[28]

Clinical experience shows that practically every organ of the human being is subject to infection with *Spirochæta pallida*, but some organs undoubtedly escape infection in a very much higher percentage of cases of syphilis than other organs. The prostate gland for example, is rarely involved insofar at least as clinical and pathological changes are concerned whereas the testicles are frequently infected; syphilis of the seminal vesicles has not so far been described. The skeletal muscles, likewise, usually escape infection unless areas of local resistance due to tissue injury predispose to localization of *Sp. pallida*.

It is possible, therefore, that during the spirochetemic stage of syphilis spirochetes are unable to localize in some tissues because these possess a sufficient degree of resistance to prevent invasion or, are lacking in suitable pabulum. The nature of this resistance, however, is unknown but one thinks of factors unfavorable to the nutrition of spirochetes or of spirocheticidal metabolites in this connection rather than destruction by phagocytosis or spirocheticidal antibodies.

It is possible but scarcely probable that allergic sensitization of the tissues of the central nervous system or of other organs may favor the final localization of spirochetes in such tissues during chronic infections. Under these circumstances foci of *Spirochæta pallida* in the lymph-glands, myocardium, etc., may elaborate protein products capable of sensitizing other tissues. If such occurred it is readily understood how spirochetes brought to these tissues by the blood would find a favorable reception and soil for localization owing to the well-known affinity of sensitized tissues for the antigen. In this connection it may be mentioned that one type of reaction sometimes following the administration of arsphenamin has been ascribed to the effects of *Spirochæta pallida* products suddenly released through the disintegration of the parasites by the medicament upon sensitized tissues. Likewise the occurrence of the *luetin* skin reaction lends weight to the supposition of sensitization in syphilis; but recent investigation by Stokes,[29] Greenbaum and myself[30] have shown that the skin reaction to luetin in syphilis may not be allergic at all, but an expression of heightened reactivity

of the skin in this disease to the injection of most any substance including the culture medium employed for the preparation of *luetin*.

Relation of the Question of the Plurality of Strains of Spirochæta Pallida to the Treatment of Syphilis.—I have briefly reviewed the evidence for and against the theory of plurality of specific tissue strains of *Spirochæta pallida* because the question is not without much interest and importance in relation to the treatment of syphilis. This relation refers not only to the admitted difficulties attending the amelioration if not the cure of neurosyphilis but more importantly to the influence of inadequate treatment of syphilis in its early stages upon the development of visceral syphilis with special reference to neurosyphilis.

It is still too early to express any opinion concerning the influence of the treatment of syphilis with arsphenamin and allied products upon the development of paresis, tabes and other forms of neurosyphilis. An interval of many years must elapse before material sufficiently large to be of value can accumulate. As shown by Mattanschek and Pilcz,[31] about 4.67 per cent. of syphilitics treated with varying degrees of thoroughness with mercury and iodids develop paresis, while 2.73 per cent. develop tabes. In their experience the majority of paretics were individuals in whom the course of syphilis appeared to be mild, regardless of the kind and amount of treatment given; no relation could be shown between thoroughness of treatment and the time of onset of paresis, but the percentage of syphilitics developing paresis and tabes was much less among those receiving thorough and intermittent treatment than among those receiving little or no treatment. Moore and Kemp also found that with regard to the syphilitic spouses of neurosyphilitics studied by them, none had received any previous treatment. It would appear, therefore, that early and adequate treatment of syphilis safeguards some against neural infections; this subject is discussed, however, with more detail in Chapter LXIX.

Thorough treatment of syphilis in its early stages is required for the prevention of neural infection; inadequate treatment may actually increase the chances of this dreaded accident. As previously stated the experimental work of Brown and Pearce has shown that treatment of the rabbit with testicular lesions of syphilis, insufficient to effect complete cure but with suppression of the local lesion, results in favoring the dissemmation of the spirochetes. Apparently the local lesions produce immunity principles or in some manner influence the spirochetes, tending to delay or prevent the progressive involvement of other organs and tissues and anything which diminishes this local reaction short of complete destruction of all parasites may actually favor the general distribution of the organisms. Keidel[32] has recently stated that in human syphilitics a similar phenomenon may be operative and that the occurrence of neurosyphilis or syphilis of other internal organs bears a relation to the occurrence and degree of the local reaction (chancre and secondary lesions) as well as to insufficient and inadequate treatment, the latter serving only to suppress these local lesions, without complete destruction of all spirochetes, and thereby doing more eventual harm than temporary good.

Some have claimed that the difficulties attending the treatment of neurosyphilis and especially paresis and tabes, may be further evidence of the plurality of strains. It is difficult, however, to understand how and why this should be the case; the acceptance of such a theory presupposes that the neurotropic strain or strains had acquired a fastness or resistance to the usual antisyphilitic remedies in common use. It is possible and indeed probable, that the spirochetes acquire some degree of fastness during treat-

ment owing to the fact that many cases of neurosyphilis are under treatment for long periods of time, but this possibility has no bearing at all upon those cases of neurosyphilis infected at a time antedating the introduction of arsphenamin and allied products in the treatment of syphilis, which includes a large percentage of the cases of paresis and tabes encountered at the present time. But it cannot be denied that in the future the transmission of spirochetes rendered resistant to organic arsenic and even mercury by long continued treatment of the syphilifer, may complicate the chemotherapy of the disease.

Just why the chemotherapy of paresis and tabes presents more difficulties than other types of visceral syphilis is difficult to state but is probably due to the parenchymatous position of the organisms. After all the same difficulties may exist under similar conditions in other of the internal organs, although evidences of our failure to improve or cure the lesions and symptoms are not as evident as in paresis and tabes, since the tissues or organs may be of lesser physiological importance than the brain and spinal cord.

In the treatment of rabbit syphilis I have so far found no evidence of variation in resistance to the curative activities of arsphenamin and neoarsphenamin among three different strains of *spirochæta pallida*, one of which was the original Nichols-Hough strain isolated in 1912 from the spinal fluid of a case of secondary syphilis developing a neurorecidive reaction after the injection of arsphenamin. Indeed, there is quite a uniformity of results among European and American investigations regarding the minimal curative doses of these compounds for syphilis of the rabbit (see Chapter XXIII). Doubtless the results are based upon work conducted with different strains. There is, therefore, absolutely no laboratory evidence today indicating that one strain of *Spirochæta pallida* is more difficult to destroy with arsphenamin and neoarsphenamin than other strains, except a strain in my laboratory which Klauder rendered slightly arsphenamin-fast or resistant by a series of transfers with the definite purpose of determining whether or not *Spirochæta pallida* could be rendered arsphenamin-resistant in the living animal.

Whether or not we accept the theory of plurality of strains of *Spirochæta pallida* or believe as I do that the whole question is one of variations in the inherent virulence and invasiveness, generalization and localization of organisms being influenced and modified by the degree of local reaction after infection and by predisposing tissue injuries and trauma, the lesson in relation to chemotherapy is plain. We must treat the disease as early as possible and in as energetic a manner as toxicological effects will permit. Furthermore we must insist upon a minimum amount of treatment for all cases regardless of clinical evidences of apparent cure and finally we must adopt rigid clinical serological and other criteria of cure. In other words, we must cure the syphilitic as rapidly as possible and we should advise and caution our patients regarding the probable influence of trauma and tissue injury as factors exerting some influence upon the subsequent localization of the parasites while their complete destruction is being attempted.

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CHAPTER XXXVII

IMMUNITY IN SYPHILIS IN RELATION TO TREATMENT AND PROGNOSIS

The subject of immunity bears an important relation not only to syphilitic infection but likewise to the treatment and prognosis of the disease. This is especially true in relation to the mechanism of reinfection, superinfection and the cure of acquired and congenital syphilis.

Natural Immunity in Syphilis.—In the first place it may be stated that natural resistance or immunity to syphilitic infection probably does not exist among human beings. Numerous instances recorded in literature purporting to show the existence of individual immunity are more likely to be explained on the basis of accidental and fortuitous escape from infection due to intact epithelial barriers of the skin and mucous membranes or to the existence of a latent but unsuspected syphilitic infection. As will be shortly discussed, the presence of syphilis and even of extremely latent syphilis associated with otherwise good general health may apparently protect against superinfection and thereby explain what may otherwise be regarded as an example of natural immunity. Races, however, vary somewhat in susceptibility to syphilis; among those peoples in whom the disease is first introduced syphilis is frequently more virulent than usual. The Negro race also appears to possess a somewhat better defensive mechanism against *Spirochæta pallida* after infection has occurred.

Syphilis does not exist naturally among the lower animals. It must be stated, however, that venereal spirochetosis of rabbits bears a close resemblance to human syphilis and the causal organism *Spirochæta cuniculi*

resembles *Spirochæta pallida* very closely, but all inoculation tests of human volunteers with *Spirochæta cuniculi* conducted by Levaditi and Marie[1] have proven negative. Evidence at present indicates that *Spirochæta cuniculi* is a separate organism.

With the exception of rabbits and monkeys, the lower animals appear naturally immune to *Spirochæta pallida*, although a few investigators claim having successfully produced syphilitic keratitis in cats, dogs and sheep. My own attempts to produce syphilis in white rats, guinea-pigs and dogs by testicular inoculations with virulent strains of *Spirochæta pallida* from rabbit and human lesions have always failed. In my laboratory Klauder has endeavored to break down the resistance of rats and especially of the lymphocytes by preliminary exposure of the testicles to *x*-rays, but without success. The nature of this natural immunity is unknown. Weiss and Wilkes have found that *Spirochæta pallida* can be cultivated *in vitro* in the various tissues of the rat and guinea-pig and that the sera of these animals are almost devoid of antibodies for culture *Spirochæta pallida*. Normal unheated rat serum rarely agglutinates *Spirochæta pallida* in dilutions higher than 1:4 and guinea-pig serum seldom higher than 1:8 and both are apparently free of spirocheticidal substances. Normal rabbit serum may agglutinate as high as 1:20 but is almost free of spirocheticidal activity; these agglutinins, however, are not protective as shown by the ease with which the testicles of rabbits may be infected. There is something in the tissues of the rat, guinea-pig and other lower animals directly destructive to *Spirochæta pallida* although when these tissues are removed and emulsified under sterile conditions the organisms can survive and proliferate in the suspension of cells. Probably the wandering cells and more especially the lymphocytes are the protecting agents, although no evidences of phagocytosis were discovered in these experiments.

Among human beings the epithelium of the skin and mucous membranes together with the surface secretions appear to be the chief factors of resistance. As stated in Chapter XXXV, it is possible, however, that on mucous membranes at least, virulent strains of *Spriochæta pallida* may gain access to the interepithelial and subepithelial lymph spaces without a previous break in the layers of cells, since Reasoner has shown the infection of rabbits by application of spirochetes to the mucosa of the genital tract and Brown and Pearce, infection of the eyes of apparently normal rabbits by dropping the parasites into the conjunctival sacs.

Doubtless, however, there is an important relation between numbers and virulence of *Spirochæta pallida* and infection of human subjects. It is entirely likely that some individuals are actually invaded by *Spirochæta pallida*, but escape infection due to the destruction of the parasites by secretions or the body fluids although these defensive agents are feeble at best and probably ineffective in the presence of many highly invasive spirochetes. All of the surface tissues are susceptible and chancres have occurred on practically every portion of the skin and contiguous mucous membranes.

After infection has occurred, striking variations in individual resistance are easily apparent. As previously mentioned the negro appears to possess a better developed defensive mechanism although his escape from tertiary lesions may be explained on grounds other than the development of an actual immunity. The apparent escape from tertiary lesions and especially of the neuralaxis among races in the tropical countries is not now believed to be as frequent as hitherto thought, since more careful examinations have been employed. But pregnancy exerts a profound influence upon the course of syphilis in both mother and child, characterized by latency of the infection, which will be discussed shortly in more detail although it is not definitely

known at present whether latency under these conditions is due to some change in the life cycle of *Spirochæta pallida* or the development and operation of immunological agencies.

Acquired Immunity in Syphilis.—After syphilitic infection has occurred, the subsequent progress of the disease is combated by various defensive agencies. Small lymphocytes and plasma cells are quickly attracted to the foci of infection; likewise a few polymorphonuclear leukocytes and these, especially the small lymphocytes, probably produce antibodies against the spirochetes. It is also possible that plasma and cellular enzymes and especially lipases derived from the lymphocytes, exert some degree of antispirochetic activity.

It is highly probable that all or, at least most immunity principles, are produced right in and about the nests or foci of syphilitic infection. Some of these substances are then absorbed and distributed throughout the body and may effectually protect against superinfection. Nichols[2] in 1914 first showed that an active lesion in the testicle of a rabbit tended to inhibit the development of potential lesions in other parts of the body; Zinsser, Hopkins and McBurney[3] subsequently found that the resistance to superinfection was confined mostly to the infected tissues, that is, a syphilitic testicle of a rabbit could not be superinfected although the other testicle could be infected at the same or a later time.

Brown and Pearce have studied this local immunity with particular care and have shown experimentally in the rabbit that anything which reduces the local primary lesion actually tends to spread the infection. In other words, the primary lesions are looked upon as an expression of defense on the part of the infected host—a place where immunological agents are being produced which combat the spirochetes. Since *Spirochæta pallida* has already invaded the lymphatic glands of the rabbit within a few days of the time of inoculation, removal of the primary lesion by castration or its suppression by the administration of subcurative doses of arsphenamin has, in their experiments, generalized the infection. Apparently the immunological principles produced locally are absorbed and tend to protect other tissues and organs against spirochetic invasion. In the rabbit, this protection may be effective in the majority of instances except for the invasion of the lypmhatics nearest the testicular lesions which are invaded by the spirochetes before sufficient amounts of these immune principles have been produced. In the human being, these principles are probably but rarely sufficient effectually to prevent the progressive invasion of other tissues, but they doubtless exert some important influence in this direction.

It would appear, therefore, that the immunity principles of syphilis are produced mainly by the tissues in direct contact with the spirochetes. Very probably the lymphocytes are chiefly concerned in their production, but the fixed cells of the infected tissues may also participate in the process. This antibody-production occurs not only in the chancre but subsequently wherever spirochetes may locate—in the skin, heart, aorta, brain, etc. It is also probable, of course, that diffusible toxins from spirochetes are produced and absorbed that may prove antigenic for the usual antibody-producing tissues, which we regard, principally, as the bone marrow and the lymphoid tissues in general.

Rôle of Immunity in the Cure of Syphilis.—Unquestionably these immunity principles exert an important rôle upon the progress of syphilitic infection. It is possible that they may influence and control in a large part the distribution of spirochetes after the primary lesion and are doubtless important factors in the production of latency in syphilis. This immunity

appears to be developed more completely in some individuals than in others, and the negro is sometimes apparently more fortunate in this respect than others. Pregnancy likewise appears to aid in the production of these protective agencies.

Unfortunately, however, we do not know how to increase the production of these substances in human syphilitics for therapeutic purposes, but I am prepared to believe that the administration of vaccines of *Spirochæta pallida* may be of aid in this direction and even non-specific protein agents, as intramuscular injections of sterilized milk or some similar protein substance by intramuscular or intravenous injection. Off hand, one would surmise that the local primary lesion of syphilis should be left alone in order to obtain the maximum protection for the balance of the tissues and organs to be derived from the production of the immunity principles in this area, but these immunological agents are seldom if ever capable of curing syphilis and, since we now have in arsphenamin, neoarsphenamin and allied products substances capable of bringing about complete destruction of all spirochetes, the indications are to treat syphilis as soon as possible after infection. But the treatment should be energetic and sufficient. As shown by Brown and Pearce, subcurative doses in the rabbit may actually aid in spreading the disease and the lesson to be learned from their experiments is of tremendous importance in relation to the treatment of human syphilis and especially syphilis in the early stages. Fortunately in the majority of instances syphilitics in the clinical, primary and secondary stages are generally in good general health permitting the energetic administration of arsphenamin or neoarsphenamin and these should be administered as soon as possible and in adequate amounts.

Syphilis is not self curative, except possibly in exceptional instances. Even though its victims may be in apparent good health, it can usually be shown that the infection is only latent and that active lesions may be developed at later periods. In the aged and in those badly crippled with syphilitic or other chronic lesions reducing tolerance for antispirochetic remedies in adequate doses, it may be well not to disturb this latency and to leave well enough alone; but the immunity principles alone cannot be expected to cure human syphilitics and in the early stages, at least, the parasites should be attacked with vigor, as the complete cure of syphilis is dependent not upon the production of immunological agents but upon the administration of adequate amounts of antisyphilitic drugs.

Antibody-production in Syphilis.—What is the nature of the immunological principles produced mainly in the foci of syphilitic infection and probably in most part by the lymphocytes? Are they antibodies belonging to those already known in a general way in bacterial diseases as the antitoxins, opsonins, agglutinins and cytolysins?

The only serum antibodies so far discovered in syphilis are agglutinins and spirochetolysins. In 1913 I first discovered agglutinins for culture *Spirochæta pallida* in the sera of immunized rabbits and in human syphilitics,[4] and this discovery was soon confirmed by Nakano,[5] Kissmeyer[6] and by Broadwell, Matsunami and myself.[7] But Zinsser and Hopkins[8] have shown that agglutinin production for virulent spirochetes in rabbit and human lesions was much less than for avirulent cultures which reduced any significance to be placed upon the presence of agglutinins in the blood of human syphilitics in relation to immunity in this disease.

On the basis of investigations prior to 1911 with emulsions of tissues rich in spirochetes, Neisser and Bruck expressed the opinion that parasiticidal antibodies are not produced in the course of syphilis. Zinsser and Hopkins[9] have shown that the sera of rabbits and sheep immunized with a pure culture of *Spirochæta pallida* developed spirocheticidal antibodies for culture strains; but later Zinsser, Hopkins and McBurney[10] found that these antibodies had practically no action for virulent strains obtained directly from human

and rabbit lesions. Broadwell and myself[11] likewise found practically no demonstrable amounts of spirocheticidal substances in the fresh sera of syphilitics for a culture of *Spirochæta pallida*, but Noguchi[12] found that serum from a syphilitic rabbit destroyed culture spirochetes and more recently Eberson[13] has reported the presence of relatively large amounts of spirocheticidal substances in the sera of both syphilitic human beings and rabbits, especially in the latent stages. Eberson believes that these antibodies represent the immunological response in syphilis and are accountable for the periods of latency.

The substance responsible for the Wassermann reaction is probably also produced in most part by the lymphocytes in syphilitic exudates, as well as by the cells of the infected tissues. Klauder and myself[14] have found that secretions squeezed from primary human lesions may yield positive complement-fixation reactions many days before the blood Wassermann test yields positive reactions and our observations have been confirmed by Fuentes[15] and by Stern and Pypins.[16] The substance responsible for the Wassermann reaction, however, is not generally regarded as a true antibody and probably is not antispirocheticidal. Rather it is generally believed to be a reactionary product of the cells aptly designated by Neisser as "reagin," but its presence in the blood and spinal fluid does not run parallel with the evidences of immunity in syphilis. Eberson has observed that in latent and apparently cured cases of syphilis the Wassermann reaction may be negative when the serum is highly protective and spirocheticidal. It is commonly observed that the Wassermann reaction is strongest when the disease is most active and for these reasons the complement-fixation test conducted with the usual tissue extracts as antigens is not an indication of immunity but rather of infection. On the other hand, complement-fixation tests conducted with antigens of saline or watery extracts of *Spirochæta pallida* may be true antibody reactions, due to the presence of a *Spirochæta pallida* antibody different from the tissue "reagin" responsible for the Wassermann reaction as ordinarily conducted.

Whether or not opsonins are produced in syphilis cannot be definitely stated. In my laboratory the experiments conducted by Weiss and Wilkes have been largely of a negative character, although, owing to technical difficulties associated with opsonin experiments employing *Spirochæta pallida*, a definite opinion cannot be expressed. It is unusual to find spirochetes located intracellularly in sections of syphilitic tissue and in my opinion, phagocytosis plays but a minor rôle in resistance and immunity to syphilis.

As stated in Chapter XXXV, *Spirochæta pallida* may produce hemotoxins and other toxins in the tissues responsible for the anemia and general symptoms of syphilis, but so far no one has actually demonstrated their presence either *in vitro* or *in vivo;* neither has any one demonstrated antitoxins in the blood of normal or syphilitic human beings or the lower animals for these hypothetical toxins.

Antibody-production in syphilis, therefore, seldom if ever reaches a high degree. As previously stated, antibodies may be engendered in sufficient amounts to produce periods of latency during the course of syphilis and may even protect some organs against invasion with *Spirochæta pallida*, as expressed by Keidel[17] in reference to the apparent escape of some syphilitic individuals from infection of the tissues of the central nervous system, but they are not produced in sufficient amounts effectually to render syphilis a self-limited or self-curative disease or effectually to protect against reinfection after the cure of syphilis. Whether or not the immunity principles engendered in the local lesions and responsible for the resistance of these to superinfection are agglutinins and spirochetocidins as found in the blood, cannot be definitely stated, but very probably this local resistance engendered in the foci of syphilitic infection are antibodies of these kinds.

Nature of Latency in Syphilis.—Latency in syphilis is one of the most remarkable phases of the disease. This does not refer to the so-called primary latent period between the healing of the chancre and the outbreak of the skin and mucous membrane lesions, during which time the parasites have been or are being deposited in the tissues with the development of lesions which represent local defensive reactions in exactly the same manner

as the chancre is a defensive reaction, but rather the latency of the later stages of the disease.

Is latency due to a reduction in the virulence of *Spirochæta pallida?* Is it a manifestation of an unknown phase in the life cycle of the parasite, or is it a neutralization in whole or part of its pathogenic agencies by the antibodies discussed above? In all probability latency is due to a combination of reduction in virulence of the spirochetes associated with a neutralization of their pathogenic activities by cellular or humoral antibodies, since definite evidence of changes in the life cycle of the parasite has not been produced. But very probably antibodies are more important in this relation than reduction in virulence, because anything depressing tissue immunity and resistance like trauma favors renewed activity of the spirochetes and, as shown by Brown and Pearce,[18] the administration of subcurative doses of arsphenamin and neoarsphenamin to rabbits may so depress immunity principles in local lesions as to render these susceptible to superinfection even though the tissues still harbor living spirochetes. Furthermore we know that the administration of arsphenamin, neoarsphenamin and mercury is sometimes followed by an exacerbation of the disease with the production of a Wassermann reaction (the provocative reaction) which I interpret as the result of a depression of immunity and local resistance followed by spirochetic activity rather than an increase of virulence of the spirochetes.

Antibodies probably reduce the invasiveness of the spirochetes in the tissues and neutralize those substances responsible for the chemotaxis of lymphocytes explaining the presence of the organisms in the heart muscle and other tissues without lymphocytic and plasma cell infiltrations as described by Warthin. But the spirochetes are not dead and possess potential pathogenic powers capable of action when the restraining influences are removed. However, there is probably a true reduction in their virulence in addition to a constant neutralization of their pathogenic agencies brought about by the effects of these antibodies.

In addition it is highly probable that tissue changes and especially the production of fibrous tissue play some part in the production of latency. In large lesions of the gummatous type the fibrous tissue and especially the vascular sclerosis tend to reduce and cut off nutrition leading eventually to tissue necrosis and the death of spirochetes. Walls of lymphocytes and plasma cells and especially of fibrous tissue may just as effectually prevent the local spread of infection or the entrace of spirochetes into lymphatic and vascular channels as in tuberculosis and prove factors of considerable importance in the production of latency and self limited distribution of syphilitic lesions.

Limitation of Latency in Syphilis.—After immunity has been acquired sufficient for the production of latency of varying degrees and duration, why may the disease spring into renewed activity at later times? Why is not the immunity lasting and sufficient for maintaining a fair degree of good health for an indefinite period?

In the first place, it is possible that the spirochetes gradually acquire a "fastness" or resistance to the antibodies just as we definitely know that bacteria may acquire a resistance to serum antibodies. If such occurs in syphilis it is readily understood that in time a race of spirochetes will be produced in the patient which are no longer held in check by the immunity principles but which proceed with the invasion and infection of new tissues and organs.

Furthermore, there can be no doubt that tissues injured by trauma are less able to resist invasion by the spirochetes than normal tissues and, under

these circumstances, spirochetes which have had their virulence reduced by antibodies are still capable of infecting these local areas of reduced vitality and resistance.

It may be that the immunity is gradually reduced in the long periods of latency if the degree of antibody-production is in ratio to the degree of spirochetic activity. Finally a point may be reached at which the antibodies are greatly reduced but without complete eradication of the parasites and, the balance between the two now being disturbed in favor of the latter, the surviving spirochetes may spring into renewed activity as the result of the administration of drugs, an intercurrent infection or some kind of tissue injury and proceed with the invasion and infection of new tissues with little or no restraint. This may be and usually is followed by renewed antibody-production, the cycle of latency and renewed activity of the infection being repeated sometimes many times during the lifetime of the syphilitic subject.

Influence of Pregnancy upon Syphilitic Infection.—In connection with this discussion of immunity in syphilis and especially in relation to the nature of latency, the influence of pregnancy upon the course of syphilis is deserving of special attention. It is a common and well established clinical experience that syphilitic mothers develop extreme and oftentimes prolonged periods of latency during which time a positive Wassermann reaction may be the only sign of infection. Likewise, pregnancy appears to reduce the incidence of neurosyphilis as compared with its incidence among men and childless women. Furthermore, this latency is sometimes transmissible to the child in the uterus, because in congenital syphilis the lesions and symptoms of the disease may not become clinically apparent for many years after birth.

Brown and Pearce[19] have shown very clearly that the reaction of the rabbit to a genital inoculation with *Spirochæta pallida* practically coinciding with conception differs very decidedly from that of the normal animal inoculated in the same way and that this difference extends through the period of pregnancy and well into the period of lactation. The differences noted were of two kinds: ordinarily, it appeared that the defensive mechanism of the pregnant animal was capable of opposing a resistance to inoculations performed at the time of conception such that little or no clinical signs of infection appeared—a condition possibly analogous to that upon which Colles' law was founded. In other instances, however, slight local lesions and marked constitutional disturbances were produced which suggested an ineffectual resistance to the infection or a breaking down of the defensive mechanism.

Gartner[20] has recently stated that on the basis of the incidence of paresis over a period of years in a population of about 38,000,000 Germans, the ratio of paretic men to women was 3.43:1 and suggests, in common with the opinions of Fournier and others, that this difference is largely due to the higher incidence of syphilis in men than in women, together with the greater chances for tissue injuries and such predisposing factors among men. But Moore[21] has recently shown that in Baltimore clinical neurosyphilis is about three times as frequent in men as in women and that, while the nervous systems of the two sexes are invaded with equal frequency in the early stages of syphilis, as shown by the relative percentages of spinal fluid abnormalities, the course of syphilis is markedly altered by the simultaneous occurrence of pregnancy and that pregnancy is the most important point of difference in the ordinary life history of the two sexes and an important factor in altering the course of syphilis in the woman. Indeed, if it were not for the danger of transmitting the disease to offspring, one could conclude from these laboratory and clinical studies that pregnancy is a therapeutic measure of con-

siderable value, insofar, at least, as concerns the production of latency and the limitation of dissemination of infection.

Just how pregnancy influences syphilitic infection is unknown. Are changes induced in the life cycle of the spirochetes? There is no morphological evidence of such in animal experiments and the examination of syphilitic placentas and fetuses. Is antibody-production increased in the mother? If so, are the antibody producing tissues stimulated by metabolic changes coincident with pregnancy and lactation? Are antibodies produced in the placenta and possibly in the fetus which are transmissible to the mother and reenforce her antibody mechanism? Very probably changes in the syncytial and placental tissues of this kind are the chief factors. The evidence at hand indicates that the mother is less extensively infected than is general in males and in non-pregnant females, and for this reason there is no reason for believing that antibody-production in the pregnant individual is greater than usual if we accept that antibody-production primarily occurs in syphilitic foci and, therefore, is roughly parallel with the degree of dissemination of the spirochetes and the number and size of syphilitic lesions. Rather it would appear that the enhanced immunity of the pregnant subject is due to a re-enforcement and, if such is true, one naturally suspects that the new tissues of pregnancy are actively concerned in the process, since it has been shown that they are frequently infected. On the other hand pregnancy and lactation may increase the invasiveness, virulence and general pathogenic properties of the spirochetes suggesting that the immunity mechanism may be depressed by some accident. Either the influence of pregnancy is exerted through the medium of an influence upon antibody-production or through some other agencies connected with the pregnant state and operative very early in pregnancy which influence the pathogenicity of *Spirochæta pallida* and generally in the direction of a reduction of these properties.

Laws of Colles and Profaeta in Relation to Immunity in Syphilis.—Colles believed that the apparently healthy mother of a syphilitic child escaped infection by reason of acquiring an immunity during pregnancy and that she could nurse the child without danger of infection. Profaeta believed that the apparently healthy child of a syphilitic mother likewise acquired an immunity and that there was little or no danger of it contracting syphilis from the mother. We now know, however, that the mother in the first instance and the child in the second are frequently infected with syphilis in extreme degrees of latency, a positive Wassermann reaction being the only sign of infection.

Doubtless the latency of syphilis in both mother and child is due to the influence of pregnancy as discussed above. As previously stated, the nature and mechanism of this reduction in the virulence and activity of *Spirochæta pallida* associated with pregnancy are unknown, but it apparently is associated with an increased antibody-production which gradually reduces the virulence of the parasites and restrains the production of new lesions and the progression of old ones. Whether pregnancy stimulates the antibody-producing tissues or the increase of antibodies is due to their production in the new tissues of pregnancy, cannot be stated, although in my opinion the latter is the more probable mechanism.

At any rate, the apparently healthy mother of a frankly syphilitic child is to be regarded as a subject of latent syphilis and the latency may be so extreme that even the Wassermann reaction may be consistently negative for long periods of time. Personally I cannot believe that it is generally possible for a woman to give birth to a syphilitic child and escape infection and I believe she is a fit subject for treatment. Her disease is latent and may remain so for many years but tertiary lesions are apt finally to develop.

Whether the child of a syphilitic woman is always syphilitic is much less certain. For example, the mother may have been infected during the last few weeks of pregnancy and it is possible under these circumstances that the child may be born before infection occurs by way of the blood stream. Furthermore, I believe it is possible for a child conceived and born during the latent stages of infection of the mother to escape infection because it is always possible that during the entire period of pregnancy the lesions in the mother may be so latent that spirochetemia does not occur and it is apparent that the child in the uterus can be infected only by way of the maternal blood. Certainly the most rigid clinical and serological examinations of the children of syphilitic women frequently fail to elicit the slightest evidence of syphilis, although it is to be admitted that one cannot be absolutely sure of freedom from infection, as it is well known that the disease of the child may be so latent that lesions do not develop until very late in childhood and, indeed, not until adult age has been reached. From the standpoint of treatment, I believe that every child of a woman presenting active lesions of syphilis during pregnancy or, in the absence of these, a persistently positive Wassermann reaction, should be regarded as syphilitic and treated as such for a time at least despite negative evidence of infection. If the child is born during the late years of syphilis in the mother, when the latter shows no clinical evidences of the disease and yields persistently negative Wassermann reactions, I believe that one may safely conclude that the child does not require treatment if it is in good general health; it is possible however, that extremely latent infection may be present under these circumstances and especially if it is the first child.

Superinfection and Reinfection in Syphilis in Relation to Immunity and Cure.—By *superinfection* in syphilis is meant the successful reinoculation and infection of a human being or lower animal already syphilitic; this added infection may be with spirochetes from the syphilitic's own pre-existing lesion or lesions, in which case it is called *auto-inoculation*, or, by spirochetes derived from the lesions of some other person or lower animal. By *reinfection* is meant the production of a brand new attack of the disease after the preceding syphilitic infection has been presumably completely cured and eradicated. The difference in meaning of the two terms is dependent, therefore, upon whether syphilis is curable and if so, whether the completely cured syphilitic is vulnerable to a new infection with *Spirochæta pallida*. Regarding the cure of syphilis there are decided differences of opinion which will be later reviewed; likewise different opinions are held regarding the questions of superinfection and reinfection in syphilis, although we now have sufficient data on superinfection to discuss the question with considerable accuracy without prominently bringing up the question of curability.

As soon as *Spirochæta pallida* has gained access to the interepithelial and deeper lymph spaces and begun to bring about tissue changes, infection has occurred even though there may be no visible evidences of the occurrence. From this time on until the chancre is apparent, superinfection is readily possible. After the chancre is well developed, superinfection is still possible for a matter of a few days but rarely for more than three weeks; the superinfection may be caused by spirochetes from the chancre and producing other chancres in the immediate vicinity called autoinoculation which occurs in 20 to 30 per cent. of primary syphilis in men, or, the superinfections may be chancres of the finger, lips, anus, etc. During the clinical secondary stage superinfection practically never occurs either accidentally or by experimental inoculation insofar as can be determined clinically; it is possible, however, that superinfection may occur at this time but without the production of a

chancre, the lesion at the site of inoculation being only a papule, as maintained by Finger and Landsteiner,[22] or, indeed, possibly without any discernible local changes at all at the point of inoculation. During the latent and tertiary stages superinfection is only occasionally successful, if the criterion of success is taken as the development of a chancre; here again Finger and Landsteiner claim that superinfection is more frequently successful than surmised, but that the local lesion does not resemble a chancre but an ulcerating tertiary type of lesion similar to those presented by the patient.

This resistance to superinfection developing early in the primary stage, reaching the acme during the secondary stage and diminishing during the latent and tertiary stages, is ascribed to the influence of immunity and, as stated by Neisser, only syphilitics possess this immunity or resistance to infection with *Spirochæta pallida*. There can be no doubt that it exists and there are no valid reasons for disputing the rôle of acquired immunity as the mechanism concerned, but whether this immunity is as absolute or as frequent as current opinions indicate is much less certain. John[23] has collected quite a large series of cases from the literature of apparently successful superinfections, although some of these may have been true reinfections of cured syphilitics. According to Levaditi and Marie[24] infection with a so-called dermotropic strain of *Spirochæta pallida* does not immunize against a neurotropic strain, and the reverse; if this is true it must be that Finger and Landsteiner are correct in their belief that superinfection is more common than indicated by clinical observations. If, on the other hand, superinfection is as rare as commonly supposed after the primary stage of syphilis, this immunity is to be accepted as evidence of the unity of the *Spirochæta pallida* unless Levaditi and Marie are in error in their statement that one strain does not immunize against the other.

Naturally one wonders why a syphilitic is susceptible to extensions and exacerbations of his own infection while apparently immune to superinfection with spirochetes introduced from without. If there is but one *Spirochæta pallida*, although different strains of this one parasite of varying virulence, why should acquired immunity principles be sufficient for protecting against superinfection and yet insufficient for preventing the infection of new tissues by the spirochetes in the body? It may be a question involving numbers of spirochetes in that the immunity is sufficient for protection against the relatively few organisms applied from without while inefficient against the many that are within. Or it may be, as I personally believe, that the spirochetes within have acquired a "fastness" or resistance to these antibodies as they may to drugs, and for this reason are not as readily and effectually controlled or destroyed as spirochetes introduced from without. Finally, it may be in part a quantitative reaction in that during the latent stages superinfection does not occur because the antibodies are at a maximum, whereas during the less latent or mildly active periods in the later stages of the disease superinfection is possible but without the development as a chancre, since the local primary lesion of the superinfection is modified by the antibodies already present in the body fluids.

Does true reinfection occur in human syphilis? That is, may an individual recover from syphilis with complete eradication of all spirochetes from his tissues and then contract a new infection? In the first place, it is of course impossible at the present time to be absolutely certain of the cure of syphilis of human beings in the sense of complete eradication of all parasites from the tissues, but I know that rabbits with testicular lesions can be absolutely cured with arsphenamin and neoarsphenamin and that after a relapse of two to six weeks may be successfully reinfected. Human beings known to have

syphilis have undoubtedly contracted true second attacks of the disease after the treatment of the first; an increasing number of such cases are being reported in which it is certain that the new chancres are not recurrent chancres (chancre redux) or tertiary lesions. But are such cases to be accepted as evidence that the first syphilitic infection was completely eradicated or, are they in some instances at least, merely superinfection, that is, brand new infections grafted on to the preceding one and contracted because the immunity was insufficient to afford protection?

For example, Lipschutz,[25] in 1917, was able to collect 357 cases of apparent reinfection recorded in the literature, but since the majority were reported before our modern methods of diagnosis and treatment, it is questionable whether all were true cases of reinfection. But more recently White,[26] Schweitzer,[27] Schamberg,[28] Klauder,[29] Bechet,[30] Williams,[31] Thibierge,[32] Boas,[33] Reasoner,[34] Driver[35] and others have reported cases conforming more nearly to the conditions laid down by Driver.

It is commonly stated that a new infection occurring in an individual known to have had syphilis is a sure sign of the complete cure of the disease. But it is not now permissible to accept this dictum. Brown and Pearce,[36] for example, have shown that syphilitic rabbits treated with subcurative doses of arsphenamin and neoarsphenamin may still be reinfected or rather superinfected and I have been able to confirm their results; indeed, subcurative doses of arsphenamin and neoarsphenamin sometimes increases the susceptibility of these syphilitic animals to superinfection.

In other words, the question of reinfection and superinfection in syphilis is, as stated by Jacobi,[37] one of immunity and not of cure. Syphilitic individuals may escape superinfection not only because they harbor spirochetes in their tissues, but because they possess a sufficient degree of acquired immunity. Individuals contracting a true second attack of syphilis cannot be assured that they have been completely cured of the first attack, but only that the first attack did not leave them immune to the disease.

Unquestionably, however, the development of a true second attack of syphilis can be accepted as evidence that the first attack *may* have been completely cured or, at least, that but relatively few spirochetes were left in the tissues, because when there are many active spirochetes in the tissues the immunity is usually sufficient to apparently protect against superinfection. Unfortunately, the immunity does not last long after a great reduction or complete eradication of the infection has been effected by treatment; in some instances the immunity has passed away sufficiently to permit reinfection or superinfection within eight months after the cessation of treatment.

Influence of Chemotherapy upon Immunity and Antibody-production in Syphilis.—As stated in Chapter XXIII there is little or no evidence to support the hypothesis that arsphenamin, neoarsphenamin and allied compounds increase antibody-production and immunity in syphilis and that a part of their curative properties are due to these effects. As stated in that chapter, there is no direct evidence to show that arsphenamin, neoarsphenamin and allied products, mercury and bismuth, which are spirocheticidal, possess any stimulating effects upon the antibody-producing tissues; if they have any direct or indirect effects at all in this direction it is only by first acting upon the spirochetes and thereby liberating antigenic products which stimulate antibody production.

Drugs cannot be said, therefore, to increase immunity in syphilis. Indeed, the reverse may occur. Brown and Pearce have found, for example, that rabbits inoculated intratesticularly and treated with subcurative doses of arsphenamin and neoarsphenamin, when the first cycle of the reactions were

nearing their acme, were highly susceptible to a second inoculation and, although not cured of their original infection, reacted to the second inoculation with the formation of lesions indistinguishable from those of the first infection; also that *in certain instances the treatment given had rendered infected animals more susceptible to infection than the normal controls.*

It would appear, therefore, that chemotherapy with arsphenamin and neoarsphenamin, at least, may actually lower immunity to syphilis instead of increasing it and the influence of these drugs upon immunity in syphilis is of great importance because the experiments of Brown and Pearce indicate that undertreatment of syphilis is perhaps almost as serious as no treatment at all, and that a few doses of arsphenamin or neoarsphenamin may give little more than a false sense of security.

Immunity in Syphilis in Relation to Prognosis.—Naturally a most important question to the syphilitic and one that he seldom fails to discuss repeatedly with the physician is the prognosis of his disease. The subject cannot be briefly dismissed and requires consideration from several different angles which are discussed in Chapter LXXIX, but here may be summarized the influence exerted by immunological factors upon this important question.

Except in rare instances, syphilis does not appear to be a self-limited or self-curing disease. Antibodies and tissue immunity are produced, but these are seldom if ever sufficient for bringing about a complete eradication of the organisms from the tissues unaided by drugs. Sometimes the physician encounters individuals who, deluded by a false sense of security by reason of latency of the syphilitic infection and good general health, believe that exercise and other hygienic measures will be alone sufficient for effecting a cure; doubtless these are aids but we have no reason at all for believing that the immunological and other natural defensive agencies of the body are able to permanently hold syphilis in check and ultimately bring about cure unaided by adequate and judicious drug treatment.

Acquired immunity in syphilis is, however, an important factor in relation to the progress of the disease and tends to limit and prevent the invasion and infection of new tissues and organs and to prevent the progressive growth of old lesions. Immunity is mostly responsible for the latency of the disease and, if syphilis is uncurable when treatment is begun in the late stages, long periods of latency are next best, and insofar as duration of life and usefulness are concerned, may practically amount to a cure in the clinical if not in the pathological sense. In some individuals immunity is apparently acquired more promptly and effectually than in others and pregnancy is especially favorable to the acquisition of immunity in both mother and child.

The acquired immunity of syphilis generally protects against superinfection after the primary stage of the disease, although this protection is not absolute and when the numbers and pathogenicity of the *Spirochæta pallida* have been greatly reduced and especially by drugs, superinfection may occur and be possibly mistaken for the true reinfection of a cured case of syphilis. Inadequate drug treatment may likewise apparently reduce immunity to superinfection and for this reason chemotherapy bears an important relation to the immunity and prognosis of syphilis.

The acquired immunity of syphilis is of short duration and rapidly disappears after the complete cure of the disease; apparently the immunity is in large part a tissue immunity participated in by the tissues in direct contact with the spirochetes and likely to rapidly diminish with reduction in the numbers and pathogenicity of the parasites.

Immunity in syphilis influences the prognosis, therefore, largely by the rôle it plays in the production of latency, even though it is insufficient for

bringing about a spontaneous cure of the disease. Chemotherapy bears an important relation to this immunity and to prognosis, because, while adequate treatment may cure the disease, in a clinical sense, at least, inadequate treatment may reduce the immunity and fail to cure, yielding only temporary beneficial results and a false sense of security.

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CHAPTER XXXVIII

PATHOLOGY OF SYPHILIS IN RELATION TO TREATMENT AND PROGNOSIS

The question of the curability of syphilis is greatly influenced by the duration of the disease and the pathological changes produced before treatment is instituted as well as the kind and amount of treatment given, and it is my purpose in this chapter to discuss the question of treatment as influenced and modified by the pathological changes produced.

Clinical versus Pathological Stages of Syphilis.—Ricord's famous classification of syphilis into the *primary stage*, embracing the chancre and adeno-

pathy, the *secondary stage*, embracing the early cutaneous and mucous membrane lesions, and the *tertiary stage*, embracing the late fibrotic (gummatous) lesions of any tissue or organ, may still be of some service for the clinical and chronological classification of the lesions and symptoms of syphilis, but it is being gradually abandoned and is entirely inadequate from the viewpoint of pathology. Fournier's fourth or *quaternary stage*, embracing tabes dorsalis and paresis, is surely obsolete and the term *latent stage* is only of clinical sense. From the standpoint of pathology it is probable that true latent syphilis is uncommon, as the spirochetes are seldom entirely inactive but are producing lesions which, on account of small size or location, may not show symptoms and thus readily escape detection. Theoretically, at least, syphilis always demands treatment, regardless of its apparent latency and harmlessness; practically, however, treatment is not always indicated and especially if active and vigorous therapeutic measures are contraindicated by senile or advanced pathological changes in a vital organ or organs.

From the standpoint of the pathologist and chemotherapeutist, the disease is better divided into *early and late syphilis*. These terms, however, are not strictly synonymous with acute and chronic. There is probably no such thing as pure chronic syphilis as a disease from the pathological standpoint; the disease may be chronic from the standpoint of symptoms and a particular lesion may be chronic in the sense that it is fibrotic, but the same organ or tissue may show early or acute lesions alongside of the old or chronic. In other words, from the standpoint of pathology and treatment, the terms "acute" and "chronic" should be applied only to the state of a particular lesion or lesions; they are not always applicable to the disease as a whole, because a late case of syphilis may, indeed, present very acute and dangerous lesions which are fresh lesions and not merely the exacerbation of old or chronic ones.

In other words, the pathologist classifies syphilis according to the histological condition of the lesions and this classification is likewise of most value to the chemotherapeutist. The stage of early syphilis is one in which none of the lesions are fibrotic and in which vascular occlusion has not occurred; hence, chemotherapy may effectually destroy all of the spirochetes. The stage of late syphilis embraces the clinical tertiary stage in which many of the lesions are fibrotic and probably beyond the possibility of cure in the sense of destruction of the parasites and absorption of the inflammatory tissue; on the other hand, however, younger lesions may likewise develop at this time and these are vulnerable to therapeutic measures before advanced fibrosis and endarteritis have occurred.

General Pathology of Syphilis.—It is not my purpose to review here in detail the gross and microscopical pathological changes of syphilis; excellent descriptions are to be found in text-books of pathology and systematic works on syphilis. After all the pathology is relatively simple and the processes essentially similar in all organs. What occurs at the site of primary inoculation is practically duplicated wherever the spirochetes may locate, except that secondary bacterial infection does not occur as it may in a chancre, unless the lesions are located in the skin or a mucous membrane. Even in the skin the development of suppurative lesions (malignant syphilis) is influenced by the state of the patient's natural immunity or resistance to staphylococci and streptococci as well as to a lesser extent by the virulence of the spirochetes.

Briefly, these changes consist of a deposition of the spirochetes in lymph spaces; in the primary lesion or chancre this usually means the interepithelial lymph spaces of the skin or a mucous membrane, followed by invasion of

the lymph spaces of the corium. From these locations the parasites are swept into lymph channels, the lymph glands and finally the venous blood. In the smallest venules they may escape into the perivascular lymph spaces, but probably are mostly conveyed to the heart, pass the capillaries of the lungs and enter the arterial blood, by which they are widely distributed and locating by preference in the perivascular lymph spaces of the smallest arterioles in different organs and about the vasa vasorum of the large arteries themselves. What governs the distribution of the spirochetes cannot be stated; some organs usually escape while others are usually invaded. The skin and mucous membranes, the aorta and heart, testicles, brain and spinal cord are the usual seats of localization. Whether invasion is controlled by physical factors, by previous injury with consequent decrease of resistance or by inherent biologic qualities of the spirochetes, are questions which have already been discussed in the preceding chapter.

The resulting defensive reaction of the host (organ or tissue invaded) is usually tardy. This is probably due to the fact that numerically the infection is not heavy; only a few spirochetes are deposited and their presence may easily escape prolonged microscopical examination of the tissues. Furthermore, they tend to proliferate slowly. The deposition of a few virulent staphylococci or streptococci in the deeper tissues may be followed in three days or less by a well-defined macroscopic lesion, but *Spirochæta pallida* apparently requires two to four weeks before a local lesion of macroscopic size becomes apparent although microscopically a lesion may be detected after a few days.

The offensive mechanism on the part of the spirochetes probably consists in the production of a toxin which devitalizes and finally destroys the cells in closest contact and especially nerve and heart cells. It may be that this destructive substance is not a true toxin; at least no one has yet demonstrated *in vitro* a toxin for *Spirochæta pallida*, but it is apparently a toxic substance of unknown quality or a substance or process impairing the nutrition and metabolism of the neighboring cells. The defensive mechanism consists in throwing about the spirochetes a wall of lymphocytes and plasma cells followed later by fibrous tissue. If the spirochetes are not offensive, that is, are in a state of extreme latency or if their toxic or other offensive agencies are being neutralized by the body fluids and cells, positive chemotaxis and walling off may not occur, as indicated by the observations of Warthin,[1] who found spirochetes in the heart muscle and elsewhere without this admiring circle of lymphocytes and fibrous tissue.

However, walling off with lymphocytes, plasma cells and fibrous tissue is the usual procedure, but the whole focus may be so small as to escape naked eye inspection. For this reason Warthin objects and rightly so, to excluding a diagnosis of syphilis on the basis of no clinical lesions or symptoms, negative Wassermann reactions and the absence of macroscopical lesions at necropsy. Indeed, his work has shown that the questions of the true incidence of syphilis, the exact sensitiveness and value of the complement-fixation and other diagnostic reactions and the exact status of chemotherapy must await further microscopical studies of the organs and especially of the heart, aorta, testicles, pancreas and adrenals of many human subjects, including not only those known to have had syphilis during life but many as well who presumably did not.

Pathologists have long known in a general way that syphilitic lesions may be of microscopic size but Warthin has shown in a particularly clear and convincing manner that such lesions may be present when macroscopic lesions are not found at necropsy. A mistake pathologists have been making here-

tofore has been to depend upon finding lesions of macroscopic size and if such were not found at the necropsy table to state that syphilis was not present. To Warthin belongs the credit for showing the error of this practice; we cannot be sure that an organ or tissue is free of syphilis until it is examined microscopically if not for *Spirochæta pallida*, at least for the histological changes of perivascular round and plasma cell infiltration with vascular changes. One drawback, however, is the danger of interpreting all such areas as syphilitic. I am sure such is not the case as similar changes may occur in other chronic infections and, indeed, as a result of the administration of mercury and arsphenamin. For this reason I believe Warthin's incidence of 30 to 40 per cent. syphilis among his series of examinations may be too high, but it is hoped that under the stimulus of his work that the pathological histology of syphilis will receive more study and probably result in discovering histological changes as surely pathognomonic as the presence of the spirochetes themselves.

In time *Spirochæta pallida* may invade in this manner practically any tissue of the body. The resulting lesions may be small and scattered and even diffuse or localized. When fibrosis is well advanced and of macroscopic size the local lesion is designated as a gumma. But very few spirochetes survive in these gummas although they may be found at the periphery, sometimes forming new and acute lesions analogous to the daughter tubercles about a large tuberculous lesion.

As previously stated, *Spirochæta pallida* is especially found in perivascular lymph spaces and in the media and adventitia of the larger vessels. The resulting lymphocytic and plasma cell infiltration followed by fibrosis naturally leads to the production of a chronic arteritis which may so thicken the walls as to encroach on the lumen and result in obliteration (arteritis obliterans). In gummas this may result in cutting off blood supply with consequent caseation and liquefaction necrosis brought about by the digestive activities of the cellular proteases from the dead cells. If an iodid is being administered at this time, the process of softening is hastened probably because the iodin neutralizes the restraining influence of the antiferment, regarded by Jobling and Petersen, as the unsaturated fatty acids of the plasma.

As previously stated, the cells of the organ invaded by *Spirochæta pallida* naturally suffer to some extent. If the parasites are of greatly reduced virulence they may do very little apparent damage and are surrounded by but few lymphocytes. As a usual rule, however, local destruction of cells occurs and especially when the spirochetes locate in the heart, brain, spinal cord and pancreas. This destruction is probably primarily due to toxic substances emanating from the spirochetes; later the fibrous tissue about the focus and especially that in the walls of the blood vessels still further reduces nutrition which may eventually end in complete destruction of these cells. Depending upon the location and size of the lesion and the number of physiologically important cells involved, symptoms are produced. Located in the posterior columns of the spiral cord, for example, the lesion need not be large or involve many of the nerve fibres before its presence is made known.

Such in brief is the general pathology of syphilis, the essential processes being similar wherever spirochetes may locate. In the untreated and uncured syphilitic new lesions are probably being constantly produced, so that the early and late lesions coexist together. From the standpoint of pathology and chemotherapy we have but two kinds of lesions—the early or pre-fibrotic and the late or fibrotic lesion. Of course, every recent case of syphilis embracing the primary and secondary clinical stages of Ricord are

early cases from these standpoints, but Ricord's latent and tertiary stages refer to clinical symptoms and time only because from the standpoint of pathology and chemotherapy spirochetes are probably never entirely quiescent and in the co-called tertiary stage truly early lesions and symptoms may develop.

Difficulties of Diagnosis and the Incidence of Syphilis.—Syphilis may manifest itself clinically in a great many different lesions and especially in the late stages of the disease. Any tissue or organ may become involved and for this reason the disease concerns every specialty in the practice of medicine and surgery. No one physician can really hope to become sufficiently skilled in detecting the lesions of the disease in all cases because its diagnosis requires too much special knowledge. For example, the dermatologist may be best able to detect the skin and mucous membrane lesions, the opthalmologist the eye lesions, the neurologist and psychiatrist the brain and spinal lesions, etc., but syphilis belongs to no one specialty in medicine since it belongs to all and after the primary and secondary clinical stages have passed, may readily enough escape diagnosis by even the most skillful physician and surgeon. During the clinically latent stages and especially in congenital and maternal syphilis, these difficulties are of course greatly multiplied. Any statistics, therefore, based upon the clinical diagnosis of syphilis for expressing the incidence of the disease are apt to be too low, because the error of failing to detect syphilis clinically is greater than the chances of error of making false positive diagnoses.

Incidence based upon histories are even more misleading, not only because many persons purposely deny all knowledge of infection, but because many honestly do not know that they have become infected. This is especially true of congenital syphilis and of acquired syphilis of women.

The Wassermann reaction has greatly aided in the diagnosis of the disease and especially in its late and latent stages. This question is discussed in more detail in Chapter XXXIX because of its practical importance and value, but here it may be stated that with present-day technic even the most sensitive methods fail to detect all cases and especially cases of latent maternal and congenital syphilis. The so-called antibody or "reagin" in the blood and spinal fluid responsible for the reaction is apparently produced by the lymphocytes and other cells about the foci of spirochetic infection, and in latent syphilis the organisms may possess so little virulence that the degree of antigenic stimulation is at a minimum, with the result that sufficient amounts of this antibody are not produced to yield positive complement-fixation reactions. The error, therefore, is apt to be on the negative side, but I believe that a sensitive and acceptable complement-fixation test is at present the best single means for the detection of syphilis after the chancre stage. The percentage of negative errors cannot be accurately stated, but it is my impression that in active syphilis—that is, in cases of syphilis of any stage showing active lesions—the percentage of negative errors is less than 5 per cent. Tabes dorsalis, however, is an exception; in this syphilitic disease the active lesions may be large enough to produce marked symptoms because of their location, but too small to produce sufficient amounts of "reagin" in the blood and spinal fluid to yield true positive reactions in about 10 per cent. of cases. In other words, the clinical symptoms of syphilis depend primarily upon the location of the lesions and secondarily upon their size; positive Wassermann reactions, however, depend primarily upon the size, activity and degree of involvement of the lesions irrespective of their location.

Incidence based upon macroscopic necropsy findings is likewise in error on the negative side. The chronic lesions of syphilis of many of the internal

organs are sufficiently distinctive to permit naked eye detection by skillful pathologists, but small lesions are readily overlooked and lesions of microscopic size of course cannot be detected unless the tissues are thoroughly examined microscopically. Symmers[2] found *gross* anatomic evidence of syphilis in 314 cases out of 4880 necropsies performed at Bellevue Hospital in New York City; this is 6.5 per cent. Warthin believes that he found microscopical evidences of syphilis in 300 out of 750 necropsies performed at Ann Arbor, or 40 per cent. Symmers based his findings upon the presence or absence of lesions of macroscopic size, while Warthin based his upon the prolonged and painstaking microscopical examination of the tissues for the diffuse and localized lesions of syphilis and for the spirochetes. Warthin has emphasized, therefore, that the absence of syphilis in a cadaver cannot be based upon failure to find macroscopic lesions, but that the tissues and especially the heart, aorta, testes, pancreas and adrenals must be examined microscopically.

However, the diagnosis of syphilis by histological examination of the tissues is likewise subject to error, since the histological changes produced by *Spirochæta pallida* are not pathognomonic. Perivascular round-cell infiltration with fibrosis of the media and adventitia of blood vessels are the most constant histological changes, but similar changes are found in other chronic infections. Indeed, as found by Lucke and myself[3] in the histological examination of the tissues of rats and rabbits treated with arsphenamin, neoarsphenamin and mercury, it is possible that these compounds may produce focal areas of round-cell infiltration in the brain, heart, etc., that may be mistaken for areas of spirochetic invasion if encountered in human tissues. Of course the finding of spirochetes in the tissues may be accepted in this country as pathognomonic, since invasion during life or after death with saprophytic spirochetes is not known to occur; but it is usually a difficult and painstaking task to find these parasites in the tissues of adult cadavers with the late lesions of syphilis.

It is not possible, therefore, to express the true incidence in syphilis. Surveys based upon clinical findings, the Wassermann test and gross pathological changes indicate an incidence varying from about 2 to 20 per cent. with a general average of about 10 per cent. of the population in the United States. This figure is undoubtedly too high for some localities and decidedly too low for others, as the incidence of syphilis varies according to social conditions and practices. On the other hand, I believe that Warthin's incidence of 30 to 40 per cent. is too high for rural communities and the middle class of people generally. At any rate clinical, serological and pathological surveys have proven that syphilis is a wide spread disease involving all peoples, regardless of rank and station and of tremendous economic importance. After all, figures expressing incidence on any basis of computation are of relative value only and of no aid in the diagnosis of any one individual; suffice for us to realize that syphilis concerns practically every speciality in medicine, that our resources for clinical, serological and pathological diagnosis are limited, and that under the best of conditions we are especially likely to fail in the diagnosis of the disease during life and to a lesser extent upon examination of the tissues after death.

Pathology of Syphilis in Relation to Chemotherapy.—In the chemotherapy of syphilis it is required that the patient and not the disease shall be treated and under some conditions this may render inadvisable or impossible any attempts toward bringing about the complete eradication of all spirochetes from the tissues. For the destruction of the spirochetes, however, we must depend upon medicinal compounds of known spirocheticidal prop-

erties. Antibodies produced by the host are not sufficient for bringing about spontaneous cure in more than rare and exceptional instances. Destruction of spirochetes by the products of necrosis of gummas and other syphilitic deposits occurs only in the localized areas directly involved. Successful chemotherapy is the mainstay of treatment and success depends upon the proper administration of a medicinal that is spirocheticidal in non-toxic amounts, that is able to reach the spirochetes and to which the spirochetes are susceptible.

These conditions are optimum in the early stages of the disease—the earlier the better. The chemotherapy of syphilis in the clinical primary and secondary stages offers the best chances for complete eradication of all spirochetes, because the parasites in the blood, lymph and lymph spaces of the tissues and organs are accessible to spirocheticidal medicinals brought to the foci of infection by the blood and lymph. Naked parasites, that is, spirochetes without any surrounding wall of lymphocytes and plasma cells are most susceptible and for this reason proper treatment in the earliest stages of the disease yields best results, but spirochetes surrounded by cellular exudates are still approachable, provided fibrosis and especially fibrosis of the blood-vessels, has not occurred.

When spirochetes are first deposited in the tissues, the problem of successful chemotherapy is not greatly complicated by the question of penetrability of the medicament—that is, whether or not the compound may reach the spirochetes if administered properly in sufficient amounts. During the first few weeks or months thereafter this problem is still relatively unimportant, because we know from experiments with rabbits that spirocheticidal substances may still readily reach and destroy spirochetes surrounded by cellular exudates. But in the late lesions characterized by fibrosis and especially fibrosis of the blood-vessels, the problem of chemotherapy is complicated by the question of whether or not the spirocheticidal medicinal compound is able to penetrate these inflammatory tissues and gain access to the spirochetes. I do not believe that the question is one of blood-vessel supply and that spirochetes may locate in a tissue so poorly supplied with arteries that a medicinal absorbed into the blood is unable to reach the parasites; rather it is a question of the condition of the vessels in the diseased foci.

Fortunately *Spirochæta pallida* tends to locate at the periphery of these fibrous lesions rather than centrally and therefore, may be more readily influenced by treatment; centrally placed parasites however, may be destroyed by a lack of pabulum or by the products of tissues autolysis brought about by occluding vascular changes. For this reason it is probably not necessary for a medicinal compound to penetrate a large gumma to effect a therapeutic influence, even though it were possible to do so. From the standpoint of improvement in the chemotherapy of chronic syphilitic lesions, it seems to me that most is to be expected in the immediate future from research devoted to methods for effecting the removal of syphilitic inflammatory exudates and fibrous tissue or increasing the permeability of spirocheticidal compounds for these tissues in order to facilitate the destruction of the spirochetes and neutralization of their products. In this connection the possibilities of discovering new spirocheticidal compounds possessing greater affinity for the organisms and syphilitic exudates along the lines of vital staining are to be kept in mind as previously discussed in earlier chapters, but permeability of fibrosed blood-vessels by spirocheticidal agents remains the most important problem, in the solving of which the configura-

tion of the molecules of the medicinal rather than their size are the important factors.

Aside from the question of the influence exerted upon the spirocheticidal activity of medicinal compounds by histological changes in foci of syphilitic infection, we have for consideration the effect exerted by wide-spread pathological changes upon the toxicity of chemotherapeutic agents for the tissues of the host. It is one thing to express toxicity of a compound for a healthy animal, but an almost entirely different one for a diseased animal with alterations of metabolism and elimination. In this manner the pathology of syphilis very materially influences the chemotherapy of the disease. We may have efficient spirocheticidal agents in arsphenamin, neoarsphenamin, bismuth and mercury and any or all of these may be capable of reaching the spirochetes in the inflammatory exudates, but an individual may not be able to tolerate them in therapetiucally active amounts, or their administration may intensify degenerative lesions in certain organs and notably the liver and kidneys.

In the great majority of cases of early syphilis the general condition of the patients is such that there are no contraindications to active treatment from the standpoint of previous disease. Even in acute tuberculosis one may usually proceed with active treatment when the victim is doubly unfortunate in contracting syphilis. From the standpoint of treatment with arsphenamin and allied products and mercury, the conditions of the kidneys and liver is, however, of much importance and, when these are damaged by preceding disease, due care must be exercised in the treatment of syphilis. Likewise the treatment of syphilis accompanied or complicated by advanced endocarditis and myocarditis must be modified to meet special conditions.

The pathology of syphilis and the general condition of each patient from the standpoint of existing or previous disease may exert, therefore, considerable influence upon the treatment of the disease which may be briefly summarized as follows:

1. Early syphilitic lesions are those dating from the time of invasion of the tissues with *Spirochæta pallida* to the prefibrosis stage. That is, the parasites may be surrounded with lymphocytes and plasma cells but thickening of the blood-vessels with fibrous tissue and the production of fibrous tissue in the lesions have not occurred. Under these conditions the chemotherapeutic compound and antibodies have direct access to the parasites although hindered according to the amount of cellular exudate. For this reason spirochetes with practically no surrounding exudates are apparently most vulnerable to destruction providing they have not acquired a resistance or fastness to the medicament employed.

2. Late syphilitic lesions are those in which fibrosis has occurred and especially fibrosis of the blood-vessels. These lesions protect the spirochetes and hinder or delay the approach of spirocheticidal compounds and antibodies. It is likewise possible that all inflammatory exudates and especially those showing fibrosis, may absorb, fix or inactivate spirocheticidal substances and thereby deviate them from the parasites.

3. Large syphilitic lesions—the gummas—tend to become autosterilized by means of loss of nutrition and necrosis due to vascular occlusion, the products of autolysis and necrosis being probably destructive for *Spirochæta pallida*.

4. Syphilitic lesions, and particularly chronic lesions, may so impair the physiological functions of organs and interfere with the elimination of arsenic and mercury as to reduce tolerance for these spirocheticidal compounds. In other words the pathological changes produced by *Spirochæta pallida*

may alter the pharmacological and toxicological effects of chemotherapeutic compounds and thereby profoundly influence the treatment of syphilis in its later stages.

5. Pathological states due to other diseases preceding or coexisting with syphilis may have a similar influence upon the pharmacological and toxicological effects of antisyphilitic drugs and thereby modify the treatment of syphilis in any of its clinical or pathological stages.

Of course, it is readily understood that in the treatment of syphilis one cannot hope to restore the cells by chemotherapeutic procedures which have been completely destroyed by *Spirochæta pallida* or its products. All we can hope to do is to destroy the parasites, prevent the destruction of more cells and to some extent promote the absorption of the inflammatory exudates. Restoration of function is governed by Nature's capacity for reproduction of tissue and this is controlled in syphilis by the same laws as are operative in other types of tissue injury. Aneurysms may not disappear, nor an aortic regurgitation or the mental stigmata of paresis, although these results are commonly expected by the laity and hoped for by not a few physicians.

Finally, the possibility of chemotherapeutic remedies as arsphenamin and allied agents, mercury and bismuth, producing injuries of capillaries and thereby favoring lodgment of spirochetes in the tissues must be mentioned in the relation of chemotherapy to the pathology of syphilis. There is no evidence to show that such actually occurs, but one should not overlook the possibility of antisyphilitic drugs given in too large doses or over too large periods of time producing degenerative lesions in the liver, kidneys, heart and brain and possibly producing thereby areas of lessened local resistance favorable to syphilitic infection.

Pathology of Syphilis in Relation to Curability and Prognosis.—In Chapter LXXIX, the questions of the curability of syphilis and the criteria of cure are discussed with more detail owing to the practical importance of these subjects, than which none are more important for the victims of the disease and concerning which they seldom fail to ask of their physician an expression of opinion.

Does *Spirochæta pallida* engender such pathological changes that the disease is uncurable in the present state of our knowledge concerning treatment?

It is still too early to express a final opinion of the end results of treatment with arsphenamin and neoarsphenamin, but by all the most rigid criteria of cure applicable during life I believe that complete cures were obtained in many instances with the older forms of treatment with mercury and iodids and that the percentage of cures is probably higher at the present time with the methods of modern chemotherapy. I am absolutely sure of our ability to cure the disease in the lower animals even when treatment is delayed until chronic lesions have developed although we cannot produce in rabbits and monkeys chronic vascular and neural lesions of the degree encountered in human subjects with the late lesions of syphilis. The increasing frequency with which individuals are reported having contracted genuine new infections after the treatment of a preceding attack of the disease lends great weight to the opinion that, when syphilis is properly treated in the primary and secondary stages at least, complete cures are obtainable.

It cannot be denied that, since the advent of arsphenamin and allied products in the treatment of syphilis, many physicians and the laity alike are deluded into false impressions of complete cure by the dissipation of the external lesions of the disease by a few injections of the medicament only to

have lesions develop at a later time; furthermore, opinions are at wide variance on the questions of the kind and amount of treatment to employ, but in general terms thorough treatment over one to two years of time apparently effects complete cures in cases of early syphilis.

Warthin states that he has found histological evidences of syphilis and indeed, the spirochetes themselves, in the tissues of numerous subjects after death who in life had been treated for syphilis and were retarded as "cured." But it is well known that the treatment of syphilis is subject to wide variations in practice since it may vary all the way from a few months of treatment with pills of protiodide of mercury with potassium iodid by mouth, or treatment with iodids alone or with sodium cacodylate alone, all of which are devoid of spirocheticidal properties in the amounts commonly employed, to thorough and prolonged treatment with arsphenamin or neoarsphenamin, mercury and the iodids. Naturally Warthin became very skeptical about the question of the cure of syphilis under the conditions of his work, but in my opinion his pessimism is not warranted or generally applicable to the curability of syphilis especially in its early stages when the disease is thoroughly treated by modern methods.

The older methods of treatment with mercury and iodids, prior to the introduction of arsphenamin and its allied products, probably did fail to completely cure many cases of syphilis; the incidence of late lesions seen to-day is ample proof of this assertion. Furthermore, we know that it is difficult to cure syphilis of the lower animals and especially of the rabbit, with these older methods of treatment.

It is to be admitted likewise that syphilis is not as quickly and easily cured in the sense of complete eradication of all spirochetes from the tissues as believed when salvarsan was first introduced in the treatment of the disease. Furthermore, it is still too early to judge what may be the late or final results in cases treated with salvarsan and its allied products. Thorough histological studies are required of the tissues of subjects treated during life with these compounds, for evidences of syphilitic infection before final opinions may be expressed. But it is certain that we are much better able to cure syphilis of the lower animals with these products and have the results checked up by rigid inoculation and histological examinations and I believe that the majority of physicians especially skilled and experienced in the present day methods of treating the disease are convinced that complete cures are obtainable when adequate treatment is instituted during the early stages at least.

The complete cure of syphilis is certainly more doubtful when treatment is instituted in the late than in the early stages of the disease. Treatment must be more prolonged, although it may be milder in order to avoid tissue injury by the spirocheticidal agents. It may not be possible to sterilize the old fibrotic lesions although the fresher lesions developing in the clinically latent and tertiary stages due to continued dissemination of spirochetes into other tissues and organs, are just as susceptible to sterilization by adequate treatment as similar lesions in the early clinical stages of the disease. Furthermore, even though complete sterilization is effected in the older lesions by persistent and prolonged treatment, the results of the tissue injury may never disappear. In other words spirocheticidal compounds do not bring about the resolution and absorption of inflammatory exudates and fibrous tissue although the iodids may aid in this process; neither do they restore the cells destroyed by the infection although the removal of the spirochetes and their toxic products may permit damaged cells to recover with restoration of their functions. Spirocheticidal compounds bring about the death of the

parasites and prevent further extension of the existing lesions and the development of new ones; absorption and repair then progress along the same lines as in other forms of tissue injury due to infection.

I believe, therefore, that the complete cure of syphilis is obtainable in the majority of cases adequately treated in the early stages of the disease. In cases presenting the late lesions adequate treatment may produce "clinical cures" in a goodly proportion of individuals, in the sense that old existing lesions are held in check, new lesions cured and further extension of the disease into new tissues and organs prevented. The problem of effecting complete cures in the early stages is not usually embarrassed by general pathological changes reducing resistance and complicating therapy. In the late stages the difficulties of effecting "clinical cure" or complete cure in the sense of complete eradication of the parasites are in more or less direct proportion to the age of the lesions, their size and location and the general condition of the patient with particular reference to the state of the liver and kidneys and allergy or tolerance to arsenical and mercurial compounds as influencing the amount of treatment that may be safely administered.

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CHAPTER XXXIX

WASSERMANN AND PRECIPITATION TESTS IN RELATION TO THE DIAGNOSIS AND PROGNOSIS OF SYPHILIS, WITH SPECIAL REFERENCE TO THE KOLMER COMPLEMENT-FIXATION REACTION

Since the discovery of the complement-fixation reaction in syphilis by Wassermann, Neisser and Bruch and independently by Detre, in 1906, the test has undergone many changes, both in the theory of its mechanism and in its technical details. Despite, however, much use and abuse, it has splendidly withstood the vicissitudes of time and fortune, and, owing to an increase of our knowledge of its mechanism and technic, is proving to-day of increasing value, not only in the diagnosis of syphilis but as a guide to the treatment of the disease as well. An enormous literature has accumulated upon this subject, but much of it is worthless owing to obvious errors in technic; one result, however, has been to create a great deal of confusion, distrust and skepticism in the minds of many physicians regarding the practical value of the test. Altogether too frequently short-cut methods and various modifications masquerading under the common designation of "Wassermann test," has been used by technicians lacking in experience and skill or driven to return results in the shortest possible time, with the result that numerous errors may be committed in the way of both falsely positive and falsely negative reactions, unless due care and skill are applied in the conduct of the test.

As I hope to point out in this chapter, conflicts between the clinical and serological diagnosis of syphilis are almost unavoidable, in some cases at

least; but I believe it is the concensus of opinion among physicians of wide experience in syphilis that, when the complement-fixation test is properly conducted, the reactions are of great value not only in the diagnosis of syphilis and especially chronic syphilis in its latent stages, but as a guide and control to treatment.

Since this treatise is devoted to the treatment of syphilis rather than diagnosis, it is not required to consider in detail the serum diagnosis of the disease but a few brief remarks may not be amiss because of the great practical importance of the subject and especially Wassermann-fastness in relation to treatment.

Specificity of the Complement-fixation Reaction in Syphilis.—The complement-fixation reaction in syphilis is not a biologically specific reaction, since the "reagin" or so-called "Wassermann substance" occurring in the serum and spinal fluid of syphilitic subjects is capable of flocculating the lipoids of any normal tissue. If the reaction occurred only with lipoids of extracts of syphilitic tissues, it would be a biologically specific reaction, but this certainly is not the case. Practically, however, the complement-fixation reaction is specific for syphilis in those places where frambesia or yaws and trypanosomiasis are rare or of unknown occurrence. In other words, the "reagin" is produced only in syphilis and frambesia and possibly in trypanosomiasis, although, as previously stated, the literature on the Wassermann reaction and especially the early literature, shows that some one at some time claims to have observed positive reactions in almost all the non-syphilitic diseases of human beings.

This subject of specificity is probably the most important question of all in relation to the complement-fixation reaction in syphilis. It is imperative that serologists adopt a method which experience has taught them to regard with confidence, and especially the weakly positive reactions. There is no special merit in observing positive reactions with the sera of frankly syphilitic individuals; indeed, no more than confirming the diagnosis of fracture of a long bone by *x*-ray examination when the deformity is obvious. The complement-fixation test must detect the clinically latent and doubtful cases, and, since the sera of these may yield only weakly positive reactions, the technic should be such that these reactions may be accepted with confidence.

I believe that it is technically possible to render the complement-fixation test practically specific for syphilis in this country, since frambesia or yaws is of rare occurrence. To this end, experience and careful work on the part of the serologist are of prime importance; the best of methods cannot yield uniformly satisfactory results in the hands of inexperienced and careless workers. Altogether too much mischief has been done by short cut methods and by insufficiently trained technicians, and the physician should exercise due care in the selection of a serologist, especially if the results of complement-fixation tests are to be accepted as guiding rather than merely confirming a clinical opinion or diagnosis.

From the standpoint of technic I hope that my new method[1] will be given a fair trial. It is significant that in over 68,000 tests, including my own, in addition to those reported by Schamberg and Klauder,[2] Shivers,[3] Harper and Curtis,[4] Palmer and Gibbs,[5] Kilduffe,[6] Schamberg and Greenbaum,[7] and others, the literature having been recently reviewed by Kilduffe,[8] falsely positive reactions have been conspicuously mentioned as not having been observed.

Finally, it should never be overlooked that syphilis confers no immunity to other diseases and that the active or latent case of syphilis may present a lesion that is not syphilitic as, for example, a tuberculous ulcer in the larynx,

a cancer, etc. Not infrequently a positive complement-fixation reaction with the serum of a latent case of syphilis presenting a tuberculous ulcer is regarded as evidence of a non-specific reaction. Lesions that do not show evidences of healing after antisyphilitic treatment should always be suspected as being non-syphilitic, even though the patient has syphilis and yields a positive Wassermann reaction.

Sensitiveness of the Author's New Complement-fixation Reaction in Syphilis.—Next to specificity, the sensitiveness of the complement-fixation reaction in syphilis is a quality of primary importance. In their anxiety to avoid non-specific or falsely positive reactions, many serologists employ methods of reduced sensitiveness, being willing to "miss" many cases of syphilis in order that the positive reactions may be acceptable. I believe that this is correct in principle, but at the same time serologists should strive for a method that is as sensitive as consistent with specificity. As previously stated, the merit of the complement-fixation test is not merely in confirming a clinical diagnosis that may be obvious and sure enough, but in aiding in the diagnosis of the clinically difficult and doubtful cases and, indeed, of cases in whom the diagnosis by historical and clinical data is not possible.

I believe it is to be admitted that the history is frequently misleading; that some individuals purposely and persistently deny all knowledge of venereal infection, while a still larger group are perfectly sincere and truthful in denying all knowledge of the disease. The latter is readily understood when it is remembered that the primary lesion may be small and regarded clinically as herpes, chancroid or some other non-syphilitic erosion, and that in women the primary lesion may not be detected at all. Furthermore, extragenital lesions may not be suspected, as also latent congenital infections. I believe it is to be likewise admitted that the disease in its chronic latent stages may escape clinical detection by even the most experienced physicians and that chronic active lesions may be so small, atypical or so situated as frequently to escape detection. It has been said that the diagnosis of syphilis cannot be made by serological examinations; rather, I believe, that not infrequently the diagnosis is not possible by any other means.

Not every case of chronic latent syphilis requires treatment; there may be well-defined contra-indications and it may be wisely decided to leave well enough alone, but there can hardly be a difference of opinion on the advisability of having a method for the specific detection of as many cases of syphilis as possible, even though treatment is not given and the results are not imparted to the patient.

At best I am convinced that no serological test can ever be expected to detect all cases of syphilis. The "reagin" or "Wassermann substance" appears to be a product of the lymphocytes and other cells in the foci of infection, and, since Warthin has shown that *Sp. pallida* may be so dormant as to live in the heart muscle and elsewhere without the usual admiring circle of lymphocytes, it is hardly to be expected that sufficient amounts of "reagin" will be present in the blood. The Wassermann reaction is an index of infection and its strength bears some relation to the activity of the spirochetes. This, however, may not be appreciated clinically, because active lesions in physiologically unimportant tissues may yield few symptoms, whereas a small lesion or series of small lesions in important tissues, as in the posterior columns of the spinal cord, may produce marked symptoms with relatively small amounts of "reagin" in the blood and consequently with the production of negative or weakly positive reactions.

Interpretation and Method of Recording the Kolmer Complement-fixation Reaction in Syphilis.—A uniform method for reporting and recording

complement-fixation reactions in syphilis would greatly aid in avoiding confusion and misunderstandings in this subject; Kilduffe and others have published pleas for the adoption of a standard method.

Possibly the adoption of the usual terminology as *strongly positive, moderately positive, weakly positive* and *negative* or some modification of these terms, would aid somewhat but means very little as long as many different complement-fixation methods are in use, because what may be a strongly positive reaction with one method may be a weakly positive with another. Indeed, this is actually the case with my new method which, on account of its increased sensitiveness, commonly yields strongly positive reactions with sera yielding weakly positive reactions with my former three antigen method.

There is but little hope of ever being able to devise a terminology for expressing complement-fixation reactions in conformity with the clinical aspects or activity of syphilis, because the reactions may vary from strongly positive in cases of latent syphilis with no demonstrable lesions, to weakly positive and, indeed, negative, in exceptional cases of acute virulent syphilis.

As previously stated, the question of primary importance is whether the serum or spinal fluid of any given case does or does not give a positive reaction regardless of the strength of the reaction or the amount of serum or spinal fluid employed. This serves for both diagnosis and as a serological guide to the treatment of syphilis, and is sufficient if true positive reactions, including those that are but weakly positive, are acceptable as evidence of syphilis and in general terms as an indication for treatment.

But, if the degree of complement fixation is acceptable as an index of the degree of spirochetic activity even though the latter is not always to be evaluated clinically, something more is to be gained by measuring the strength of the reaction. Furthermore, when the fixation test is being employed as a guide to treatment, a measure of its strength is of some value, even though this may fluctuate and does not usually proceed from the positive to the negative state in a uniform manner except in cases of acute syphilis treated with arsphenamin or allied products.

In my new quantitative method each serum is tested in graded amounts after the method of Detweiler,[9] the amounts being 0.1, 0.05, 0.025, 0.005 and 0.0025 c.c. Spinal fluids are likewise employed in graded amounts as 0.5, 0.25 c.c., etc. The test requires six test tubes instead of two and the time required is slightly greater, but a method has been worked out[10] whereby these dilutions are quickly set up, and after some practice has been gained, as many as 20 to 40 sera or spinal fluids can be diluted within an hour.

This method not only yields a truer quantitative reaction than the older two-tube test, but likewise increases the accuracy of the reactions. Contrary to usual impressions, the largest dose of serum does not always yield the most sensitive reaction. One naturally supposes that to increase the delicacy or sensitiveness all that is necessary is simply to increase the amount of serum employed; but this is far from being true. In the first place the larger amounts may yield anticomplementary reactions, but even if this does not occur, something in the serum tends to interfere with complement fixation so that a large dose of serum as 0.2 c.c. may actually yield a weaker reaction than 0.05 c.c. Most serologists believe this is due to natural antisheep hemolysin, but in my opinion this factor does not explain all such reactions. Apparently some other serum constitutents, as the proteins or salts, may interfere with the fixation of complement. However, while the mechanism is in doubt the observed facts are not and in my new method, at least, 0.1 c.c. of syphilitic serum may actually yield a weaker reaction than 0.05 c.c. For this reason alone I believe it is necessary in the interests of the best work

to test every serum in at least two amounts and the added labor of using the full series of five dilutions is so slight that the complete quantitative test is very much to be preferred for routine work. I do not know definitely if the same is true of other methods, but I surmise that in all tests employing single doses of serum as 0.1 or 0.2 c.c., the same error is possible, although I have observed no evidence of this error in tests employing spinal fluids.

In my new method, therefore, each serum and spinal fluid is tested in five graded amounts, the sixth tube being the serum control. *The results are expressed according to the smallest amount of serum or spinal fluid yielding a positive reaction;* at the same time the degree of fixation after the ++++, +++, ++ and + method may be expressed for each tube showing fixation. In other words, the test yields a two-way quantitative reaction: (*a*) according to the smallest dose of serum or spinal fluid yielding a positive reaction of any degree and (*b*) according to the degree of fixation in the individual tubes. The former is the more important and more definite in results; the latter is variable because so much depends upon the human eye and what one person may read as a +++ another may read as ++. I have described a reading scale, however, which reduces this error.

According to my method the reactions are reported as follows:

Very strongly positive, if complement fixation of any degree occurs in the first four or five tubes.

Strongly positive, if fixation of any degree occurs in the first three tubes.

Moderately positive, if fixation of any degree occurs in the first two tubes.

Weakly positive, if fixation of any degree occurs in the first tube.

Negative, if hemolysis is complete in all.

At the same time, the readings on each tube showing fixation are recorded after the ++++, +++, etc. method of Citron. A few examples will suffice for purposes of illustration:

0.1 c.c. ++++
0.05 c.c. ++++
0.025 c.c. ++
0.005 c.c. +
0.0025 c.c. −

This reaction was reported as *very strongly positive* (4421−).

0.1 c.c. ++++
0.05 c.c. ++++
0.025 c.c. +++
0.005 c.c. −
0.0025 c.c. −

This reaction was reported as *strongly positive* (443−−).

0.1 c.c. ++
0.05 c.c. +++
0.025 c.c. −
0.005 c.c. −
0.0025 c.c. −

This reaction was reported as *moderately positive* (23−−−) and is an example of when the 0.05 c.c. amount of serum yielded a stronger reaction than the 0.1 c.c. dose. This, however, does not influence the general report of moderately positive.

Occasionally the reaction is about as follows:

0.1 c.c. −
0.05 c.c. ++
0.025 c.c. −
0.005 c.c. −
0.0025 c.c. −

This reaction was reported as *moderately positive* (−2−−−) even though the largest dose of serum (0.1 c.c.) reacted negatively. This occurs about once in every 200 to 300 sera and is an example of the importance of using a quantitative method.

0.1 c.c. +++
0.05 c.c. −
0.025 c.c. −
0.005 c.c. −
0.0025 c.c. −

This reaction was reported as *weakly positive* (3— — — —).

0.1 c.c. —
0.05 c.c. —
0.025 c.c. —
0.005 c.c. —
0.0025 c.c. —

This reaction was reported as *negative* (— — — — —).

Prognostic Value of the Complement-fixation Reaction in Syphilis, and the Significance of the "Wassermann-fast" Reaction.—The most difficult problem in the treatment of syphilis is the proper interpretation and management of those cases where sera yield persistently positive complement-fixation reactions in spite of active and prolonged treatment. Not infrequently many such individuals appear clinically free of syphilitic lesions and in good general health, although thorough examinations are apt to disclose evidences of syphilitic infection in the cardiovascular, central nervous or other organs. Opinions are divided, however, among syphilographers of wide experience regarding the significance of such reactions and the best means of treatment.

The *frequency* of "Wassermann-fast" cases of syphilis is difficult to compute, but the condition is relatively common and occurs probably in from 10 to 20 per cent. of cases of syphilis in clinic and private practice of to-day. Of cases of primary and secondary syphilis treated intensively and adequately with arsphenamin or neoarsphenamin, the condition is not likely to develop in more than 5 per cent., but of cases of latent and chronic syphilis, including congenital cases who have had inadequate treatment or none at all in the earlier stages, it is found that least 20 to 30 per cent. will continue to give presistently positive complement-fixation reactions after thorough courses of treatment with arsphenamin or neoarsphenamin and mercury. For example, of 458 syphilitic patients who had received twelve to twenty-nine arsphenamin injections combined with mercurial inunctions, the average being fourteen injections and ninety inunctions in eleven months, Stokes and Burman[11] found 6.6 per cent. of primary and secondary cases and 22 per cent. of latent, late and hereditary cases remained persistently Wassermann-positive.

Just when a case should be regarded as "Wassermann-fast" is a matter of individual judgment and particularly so, in view of the fact that persistent treatment of some cases ultimately results in eradicating this quality of the serum. Any case of primary and secondary syphilis yielding persistently positive reactions after the first six months of treatment described in Chapter LXXI is regarded by me as possibly "Wassermann-fast" and cases of chronic syphilis (latent and active) yielding persistently positive reactions after one year of mixed treatment can be regarded as "Wassermann-fast." These statements refer only to tests with blood serum; even after negative reactions occur with blood serum, the cerebrospinal fluid may continue to yield positive reactions as well as pleocytosis, increase of proteins and positive colloidal gold reactions. Undoubtedly the percentage of "serological fast" cases of syphilis, that is, cases showing abnormal changes in the spinal fluid as well as positive blood serum reactions, would be increased if statistics were based upon spinal fluid examinations as well as blood examinations.

The *cause* of the "Wassermann-fast" condition is naturally of prime importance in relation to both prognosis and treatment. Can the condition be possibly due to the effects of arsphenamin, neoarsphenamin and allied compounds or mercury, upon the liver or other internal organs, with the production of such substances of cellular degeneration as may result in physico-chemical changes in the serum of a character to yield positive complement-fixation reactions? As stated in Chapter XLVI, we know that these drugs are capable of producing pathological changes in the liver, kidneys, etc.,

and the subject is not to be passed over too lightly. Strickler, Munson and Sidlick[12] have asserted that the administration of arsphenamin and neoarsphenamin to supposedly non-syphilitic human subjects resulted in the production of temporarily positive Wassermann reactions while mercury was without such effects;[13] but in my experiments[14] with rabbits treated with arsphenamin, neoarsphenamin and mercury, no evidence of such reactions was found and these results have been recently confirmed by Kilduffe.[15]

It cannot be overlooked, however, that the complement-fixation reaction is frequently more strongly positive during and immediately after a course of treatment than after a period of rest. It is commonly advised to wait for two or three weeks after a course of treatment before conducting the complement-fixation test, in order to avoid securing a falsely negative reaction which is believed possible immediately after treatment. On the contrary, I have usually found the reverse; that is, the complement-fixation test frequently yields a positive reaction immediately after a course of treatment, which gradually becomes weaker and sometimes negative during a period of two to four months without specific medication. This suggests that the drugs may be responsible for the positive reacting quality of the serum through their effects upon the liver or other internal organs, but I do not believe such is the case. In my opinion these effects are due to the production of "reagin" by spirochetes actively stimulated by the drugs and that as treatment subsides the surviving spirochetes become latent or less active with a reduction in "reagin"-production.

No evidence has yet been produced to disprove the commonly accepted theory that persistently positive Wassermann reactions are due to anything other than the presence of active spirochetes in the tissues.

Just why spirochetes in these chronic latent and active lesions are so resistant to destruction cannot be stated in exact terms. The condition is frequently passed over with the statement that they are protected by fibrous tissue which, being largely free of blood-vessels, reduces the chances of medicinal compounds reaching the parasites. I believe that such may be the case, in part at least, coupled with the fact that the syphilitic mesarteritis may bring about a high degree of vascular occlusion and reduce at least the rate of penetration of spirocheticidal agents in the inflammatory tissue of infection. For this reason I believe the guiding principles of treatment in such cases should be the administration of small doses over long periods of time, along with the administration of the iodids for the possible aid they may render in aiding access of spirocheticidal compounds by the softening and removal of fibrous tissue and lymphocytic exudates.

In a thorough study of 101 cases by Stokes and Busman previously referred to, the value of thorough clinical and roentgenological examinations is emphasized and shows how frequently lesions may be detected when superficial examinations may yield to false impressions. It is commonly stated that most "Wassermann-fast" cases show syphilitic involvement of the central nervous system, but in the series studied by Stokes and Busman, cardiovascular changes were found in 44 per cent., neural involvement in 30 per cent., hepatic, splenic, and gastric lesions in 21 per cent., and other types in from 10 to 17 per cent. In this series only 10 patients presented no other evidences of syphilis than their positive complement-fixation reactions. In other words the condition of "Wassermann-fastness" of the serum is not necessarily a sign of neurosyphilis and lends no support to the theory of the existence of neurotropic strains of *Sp. pallida*. Indeed, as discussed in Chapter LXXVI, cases of neurosyphilis with "Wassermann-fast" blood reactions quite commonly show lesions in the cardiovascular and other organs

as well, as recently shown in a study by Moore and Kemp.[16] Certainly "Wassermann-fastness" is not to be regarded as a trivial condition and such cases should at least receive periodic examinations and courses of treatment for the balance of life. I am in the habit of explaining the condition and giving comfort to patients by drawing an analogy to dental caries, in that a complete "fixing up" of the teeth this year does not guarantee against the development of cavities in the future and that the best means of avoiding pain and distress is having periodic examinations of the teeth once or twice a year.

Diagnostic Value of the Precipitation Reactions in Syphilis.—I believe the mechanism of the Wassermann reaction is due to the adsorption or fixation of complement by aggregates or precipitates of lipoids of the tissue extract employed as antigen, as the result of a physico-chemical reaction brought about by the syphilitic "reagin" in the blood serum or spinal fluid. This idea is an old one and it is not surprising that numerous attempts have been made, since the original efforts of Michaelis[17] in 1907, to so alter the proportions of antigen and serum employed as to render visible to the naked eye these flocculent masses, in order to thereby simplify the serological diagnosis of syphilis. The reactions of Porges and Meier,[18] Herman and Perutz,[19] Meinicke,[20] Vernes,[21] Sachs and Georgi,[22] Dreyer and Ward,[23] Kahn,[24] etc., are reactions of this kind and the Sachs-Georgi and Kahn tests have especially attracted considerable attention.

My opinion of them in the diagnosis of syphilis is set forth in detail elsewhere[25] and is largely based upon observation of the results secured by Strumia[26] in my laboratory in a study of the Meinicke, Sachs-Georgi, Kahn and Vernes methods. I believe that these flocculation tests and particularly the Kahn test, are of distinct value as serological controls for the complement-fixation reaction in the diagnosis of syphilis, but the preparation of the antigen upon which their success so largely depends is just as intricate and important as in the complement-fixation reaction. It is true that the technic of setting up the tests is simpler than the latter, but it is likewise true that it is much more difficult to interpret and evaluate the doubtful and weakly positive reactions. With the sera of frankly syphilitic and non-syphilitic individuals, the precipitation reactions run closely parallel with the complement-fixation reactions, but, with the clinically latent and doubtful cases of syphilis in which group serological tests should be of most aid in diagnosis, the complement-fixation reaction has generally proven the superior method. Furthermore, the precipitation tests are not as successfully conducted with cerebrospinal fluids as is the complement-fixation reaction. I believe that these flocculation tests are of value, but that they require almost as much care in the preparation of the reagents as the complement-fixation test, and while it is true that they require less time, this constitutes no advantage in my opinion as long as the question of accuracy in results is involved. They cannot be made to yield quantitative reactions in the same manner as the complement-fixation test employing varying amounts of serum and are, therefore, much less useful for serological guidance in treatment. I now employ the Kahn test routinely as a control on the complement-fixation test; every once in a while the complement-fixation test yields a true positive reaction when the precipitation reaction is negative, but the reverse occurs just as frequently and both should be employed for obtaining the best results. In my experience the precipitation test is also more likely to yield persistently positive reactions during treatment, but whether these should be taken as indicating further treatment when the complement-fixation reactions are repeatedly negative, cannot be stated at the present time.

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CHAPTER XL

WASSERMANN TEST IN RELATION TO THE TREATMENT OF SYPHILIS AND AS AN INDEX OF CURE, WITH SPECIAL REFERENCE TO THE KOLMER COMPLEMENT-FIXATION REACTION

Within a few years after the discovery of the Wassermann reaction, Citron,[1] Bruck and Stern,[2] Hochne,[3] Pedersen[4] and others published observations showing that under the influence of mercurial treatment the sera of syphilitic individuals tended to yield weaker Wassermann reactions and in some instances resulted in negative reactions. With the advent of salvarsan in the treatment of the disease in 1910, interest in this subject was greatly renewed, and Nichols,[5] Nichols and Fordyce,[6] Fox,[7] Citron,[8] Weschselmann,[9] McIntosh,[10] Michaelis,[11] Muller,[12] Noguchi and Bronfenbrenner,[13] Craig[14] and others were among the first investigators calling attention to the fact that treatment with this substance sometimes resulted in producing negative mcoplement-fixation reactions along with symptomatic improvement. Of course it is not surprising that the earlier reports varied greatly in the degree of influence of treatment upon the Wassermann reaction, but they served to establish the important principle that the complement-fixation reaction in syphilis is influenced by mercury and salvarsan and constitutes a very valuable guide in treatment and a criterion of cure.

Mechanism of the Influence of Chemotherapy upon Complement Fixation in Syphilis.—While the exact nature of the substance in serum and spinal fluid responsible for complement fixation in syphilis is unknown, it is, how-

ever, directly or indirectly a product of *Spirochæta pallida* and, as stated in the preceding chapter, bears a more or less direct relation to the degree of spirochetic activity.

The administration of spirocheticidal compounds, as arsphenamin and allied products, bismuth, mercury, etc., results in a diminution of the complement-fixing substance, by reducing the numbers and activity of the spirochetes; on the other hand, however, the administration of these compounds in subcurative doses may result in a temporary increase of complement fixation by increasing the numbers and activities of *Spirochæta pallida* in the tissues. The latter is well known as the provocative Wassermann reaction and is considered in more detail in Chapter XLI.

There are no reasons for supposing that arsphenamin and allied products, bismuth and mercury are capable of themselves of producing substances in the serum yielding positive complement-fixation reactions. Arsphenamin in blood drawn immediately after the intravenous injection of this substance has no such influence, although it is possible that compounds administered in too large doses may bring about degenerative changes in the liver, kidneys, etc., and an increased anticomplementary activity on the part of the serum.

It is to be expected, therefore, that any therapeutic measure resulting in a decrease of spirochetic activity would be followed in time by a decrease in the degree of complement fixation. However, in chronic active or latent syphilis, the decrease is not regular or progressive, but is apt to be up and down; this is readily understood when it is remembered that during treatment the numbers and activity of spirochetes probably fluctuate but with a gradual tendency toward a decrease.

Specific medication in syphilis may, therefore, either temporarily increase the strength of the Wassermann reaction by temporary increase of spirochetic activity or gradually bring about a decrease by destruction of the parasites. These serological changes, however, may not bear a relationship to the clinical progress of syphilis, and for this reason some syphilologists do not accept the complement-fixation reaction as of value in guiding the treatment of the disease. I will, however, later discuss this phase of the subject.

Stage of Syphilis in Relation to the Influence of Treatment upon the Complement-fixation Reaction.—As would be expected, the earlier treatment of syphilis is instituted the better are the clinical results and the same is true in relation to the influence of treatment upon the complement-fixation reaction. Craig[15] has published statistics based upon his test in the army, showing that in primary and secondary syphilis salvarsan treatment is much more efficacious in bringing about negative reactions than in latent and tertiary syphilis and in the former somewhat better than in the latter.

As I shall shortly discuss in relation to the influence of arsphenamin and neoarsphenamin treatment upon reactions with my new test, the power of the serum for fixing complement usually decreases progressively and in an orderly manner to the negative reaction in primary and secondary syphilis, whereas in latent and tertiary syphilis the effects are more irregular and apt to be in the nature of unexpected increases and decreases with but a tendency to become progressively weaker even in those cases which may be suspected as being "Wassermann-fast."

These effects are readily understood. In primary syphilis the spirochetes have not been widely disseminated and are more readily reached and destroyed by arsphenamin and neoarsphenamin; consequently the complement-fixation reactions tend to become progressively weaker and are more profoundly influenced. Indeed, best results in the treatment of syphilis are

observed in those cases in which treatment is begun before positive serum reactions are obtained and in which, by means of adequate treatment, they never become positive. In chronic syphilis, on the other hand, spirochetes are not as readily and quickly destroyed; treatment may stimulate them in some instances followed by stronger complement-fixation reactions, or destroy large numbers at another time followed by a reduction in complement-fixing power. Apparently the degree of influence of treatment upon the complement-fixation reaction is a rough index of the degree of curative influence, although in chronic syphilis it may be possible that cells stimulated to produce the "reagin" may continue to do so for a period after all spirochetes have been actually destroyed in a focus of infection.

Degree of the Complement-fixation Reaction in Relation to Its Disappearance under Treatment.—In a general way, cases of syphilis in any given stage of the disease presenting strongly positive complement-fixation reactions before treatment is begun require more treatment for dissipating the reaction than cases beginning with weakly positive reactions. This is especially true in primary syphilis, but in chronic syphilis exceptions occur so frequently that the relation is by no means a constant one. Not infrequently cases presenting weakly positive reactions at the outset are followed by strongly positive reactions after a few doses of neoarsphenamin or arsphenamin and require more treatment for negative reactions than cases presenting active lesions and strongly positive reactions at the beginning of treatment. In acute syphilis, in either the primary or secondary stages, the physician may confidently expect negative reactions with adequate treatment more quickly if the reactions are weakly or moderately positive than if the reactions are very strongly positive; in chronic syphilis, however, and especially in the latent stages when the reactions are apt to be weakly positive, great caution is required in prognosis and it is always to be expected that, contrary to expectations, the reactions after the first few doses of arsphenamin and neoarsphenamin may be actually stronger than at the beginning followed by a progressive weakening. This is apparently due to a provocative influence of treatment and should profoundly influence the kind of treatment employed; for example, to give such a patient one or two injections of neoarsphenamin may actually do more harm than good, if one is to judge according to the influence exerted upon the complement-fixation reactions.

Technic of the Complement-fixation Test in Relation to the Influence of Treatment and Cure of Syphilis.—It is scarcely necessary to state that the technic of the complement-fixation test bears a very important relation to this subject of the influence of treatment upon serological reactions. When a sensitive method is used, it is readily understood that the reactions remain positive for a longer period of time than when less sensitive methods are employed.

For example, tests conducted after the original Wassermann technic, except that an alcoholic extract of syphilitic liver is employed as antigen instead of a saline or water extract, yield negative reactions under treatment much sooner than tests employing cholesterolized extracts. In other words, the technic employed with special reference to the antigen exerts a tremendous influence upon the reactions and it is a serious error to rely upon the complement-fixation test as a guide in therapy unless the test is known to yield as sensitive reactions as possible with specificity. From 1907 to 1910 reports on the influence of treatment upon the Wassermann reaction were based upon the use of mercury and the iodids, and the tests were usually conducted according to the original Wassermann method; the latter, in a way, was fortunate because, if the earlier investigators had employed the more

sensitive antigens and methods available to-day, they probably would not have noted sufficient influence of mercury upon the reactions to consider worth while recording their observations in literature. A few examples may better explain this very important relation of technic to the value of the complement-fixation reaction as a guide in treatment and a criterion of cure. In my former method, three antigens were employed, namely, a cholesterolized alcoholic extract of beef heart, a plain alcoholic extract of syphilitic liver and an extract of acetone insoluble lipoids of beef heart. The technic was otherwise essentially the same as the original Wassermann test except that one half amounts of each constituent were employed. The new method, however, employs a new antigen of superior sensitiveness, a primary incubation of 18 hours at 6° to 8°C., followed by ten minutes in a water-bath and numerous other technical improvements. In a series of cases in my practice treated exclusively with neoarsphenamin in dose of 0.9 gm. twice weekly, the amount of treatment required for two to five consecutively negative complement-fixation reactions were as shown in Table 5:

TABLE 5.—NUMBER OF INJECTIONS OF NEOARSPHENAMIN REQUIRED TO PRODUCE NEGATIVE REACTIONS WITH THE OLD AND NEW COMPLEMENT-FIXATION TESTS

DIAGNOSIS	OLD TEST			NEW TEST
	CHOLEST. ANTIGEN	ALC. SYPH. LIVER ANTIGEN	ACET. INSOL. LIPOID ANTIGEN	
Primary syphilis	8	3	6	11
Secondary syphilis	10	5	7	14
Secondary syphilis	13	6	9	18
Secondary syphilis	10	4	8	12
Chronic (latent) syphilis	18	11	15	32
Chronic (active) syphilis	23	14	18	41
Chronic (active) syphilis	17	11	12	26

As shown in this table Wassermann tests conducted with a plain alcoholic extract of syphilitic liver yielded negative reactions under treatment much sooner than reactions conducted with Noguchi's antigen of acetone insoluble lipoids and very much sooner than reactions conducted with a cholesterolized extract of beef heart. For any physician who desires to secure negative Wassermann reactions most speedily under treatment, I recommend the original Wassermann test conducted with an antigen of alcoholic extract of syphilitic liver, beef heart or guinea-pig heart with a warm primary incubation of one hour in a water-bath or incubator. But if he stops treatment on the basis of a negative reaction obtained with such a technic or even upon a series of such negative reactions, I am absolutely sure that the majority of his patients will subsequently develop positive reactions and otherwise show the results of insufficient treatment. If such a test is employed the antigen should be an acceptable cholesterolized extract, because this antigen yields much more sensitive reactions that are specific under proper technical conditions and guarantees a more thorough treatment than would otherwise be the case.

Positive reactions with my new method usually require still more treatment for reduction to negative, but there is not the slightest evidence to indicate that the positive reactions are non-specific and there are absolutely no reasons for believing otherwise than that the longer persistence of the positive reactions under treatment is because the method possesses a greater

degree of sensitiveness as a detector of continued spirochetic infection of the tissues.

It cannot be too strongly emphasized, therefore, that the technic of the complement-fixation test bears a very important relation to this subject of the influence of treatment upon reactions.

It is true that with sensitive methods more treatment is required to bring about a state of permanently negative complement-fixation reactions with the serum and spinal fluid than with less sensitive methods and indeed, it may be impossible to bring about this result at all with so-called "Wassermann-fast" cases of syphilis, but if a technically correct positive complement-fixation test is acceptable as evidence of the continued presence of *Spirochæta pallida* in the tissues, even though some subjects may be clinically free of lesions and symptoms, we must face the issue and frankly confess our failure in bringing about a complete removal of the spirochetes from the tissues.

It is very easy indeed for both patient and physician to be lulled into a false sense of success in treatment by negative Wassermann reactions with insufficiently sensitive methods. In such instances, however, the serum is very likely at a later time to again yield positive reactions and much valuable time may have been lost in treatment.

It may be that time will teach us that cases of syphilis clinically free of symptoms but showing persistently positive Wassermann reactions with a sensitive test are indeed cured, but there is absolutely no reason at all at the present time for believing that such is the case; indeed, Warthin has shown that just the reverse is probably true and that in such cases spriochetes may still be present in the tissues, even though the serum and spinal fluid Wassermann tests yield negative reactions. While there may be a difference of opinion on the question of having too sensitive a complement-fixation test for the diagnosis of syphilis, I cannot understand how there can be a difference of opinion on the question of demanding as sensitive a test as possible for guiding the treatment of the disease and especially if negative reactions are accepted as criteria of cure. It is understood, of course, that I refer to technically acceptable tests yielding as sensitive reactions as consistent with specificity.

For this reason I have endeavored to render my new complement-fixation test as sensitive as possible with specificity. I have had absolutely no evidence at present that positive reactions are due to any disease in this country but syphilis. Experience has shown that treatment does bring about a gradual decrease in the degree of complement fixation and ultimately negative reactions, but the amount of treatment required to effect these results is greater than required to secure negative reactions with most other complement-fixation tests. We have long since given up the idea that one or even a few injections of arsphenamin is curative for syphilis, even though sufficient in some cases for dissipating the external lesions; let us frankly confess and realize that syphilis is not as easily and quickly cured as popular ideas and opinions profess, and that the majority of us are probably undertreating the disease.

Technic, therefore, greatly influences the value of the complement-fixation reaction as a guide in therapy and especially as a criterion of cure. Physicians cannot be sure of the cure of syphilis on clinical grounds alone, regardless of their experience and skill; one or more decades will be required to properly evaluate the results of present day methods of treatment. If the complement-fixation test is enlisted as an aid in guiding therapy and for determining cure, I repeat that the responsibility of both clinician and serologist is a great one and that much harm can be done by relying upon a defective method. Better, much better, to rely on clinical judgment alone

and treat syphilis by rule of thumb than to rely upon a short cut complement-fixation test yielding too many falsely negative reactions.

Finally, until we have a standardized technic, it is almost fruitless to attempt comparing the therapeutic results secured by one investigator with those of others, because the technic has such an important influence upon the reactions and results. One physician, for example, may report that four injections of arsphenamin cure 90 per cent. of cases of syphilis in the secondary stage as based upon the Wassermann reaction; another may find that this amount of treatment does not cure 10 per cent. of his cases, and both may speak of the "Wassermann test" when the tests employed by each my differ from each other in essential technical details—sensitiveness and specificity—as night from day, even though both are based upon Bordet's phenomenon of complement fixation. It is well for physicians to dismiss from mind that a Wassermann test is the same in all laboratories and it is imperative to have tests conducted with a technically correct method yielding as sensitive reactions as consistent with specificity, when employed for guiding the treatment of syphilis and as a criterion of cure.

Influence of Iodids upon the Complement-fixation Reaction in Syphilis. Potassium and sodium iodids administered orally in the usual amounts do not appear to have any constant effects upon the complement-fixation reaction in syphilis. It is difficult, however, to express a definite opinion, because so few cases of syphilis are treated exclusively with iodids. With rabbits experimentally infected with *Spirochæta pallida* and treated with sodium iodid by daily intravenous injection of 0.100 gm. per kilogram of weight (corresponds to 100 grains per 125 pounds) over a period of eight days after the sera had acquired an increased complement-fixing power due to syphilitic infection, no evidences of any effect of the iodid upon the lesions or complement-fixation reactions were elicited. Slight variations and fluctuations in complement-fixing power occurred as would be expected, but in no instance were negative reactions obtained.

In one case of latent syphilis yielding moderately positive complement-fixation reactions, potassium iodid was given in large doses over a period of several months in the treatment of a coincident actinomycosis; a series of complement-fixation reactions with my new method showed practically no effect upon the reactions. Eight months later, when the actinomycotic lesion had been healed, treatment of the syphilitic infection was undertaken with neoarsphenamin and mercury, with a gradual reduction in the strength of the complement-fixation reactions.

There is practically no literature upon this subject, but my tentative opinion based upon the above mentioned experiences is that the iodids alone probably do not materially influence the complement-fixation reaction in syphilis. Treatment of syphilis of human subjects with protiodid of mercury may exert an appreciable influence, but the effects are to be ascribed to mercury rather than to iodid.

Influence of Mercurial Compounds upon Complement-fixation in Syphilis.—It is readily understood that the influence of medication upon complement-fixation in syphilis will necessarily vary according to the rapidity and degree of therapeutic activity. For this reason mercury influences the serological reactions much more slowly than arsphenamin and allied products.

From 1907 to 1910 all reports on the influence of treatment upon the Wassermann reaction were based upon the use of mercury and the iodids, and the percentage of cases yielding negative reactions was comparatively small. The results, however, would have been even less noteworthy at this period if the technic of the Wassermann test had yielded more sensitive reactions. With more modern methods, the complement-fixation reaction

in syphilis is gradually brought to a negative state in some cases of syphilis treated with mercury, as indicated by the reports of Best,[16] Holler[17] and others; but with sensitive methods, the influence of intramuscular injections of the salicylate are weak and much inferior to arsphenamin and neoarsphenamin, as shown by the investigations of Nelson and Anderson[18] and Goodman.[19] Indeed, the latter investigators found that salicylate of mercury had so little influence upon the complement-fixation reaction in syphilis, that they have questioned the curative activity of this substance. Thus, Nelson and Anderson found that they did not obtain a single permanent negative reaction in 50 cases of syphilis treated by ten to twenty or more intramuscular injections of mercuric salicylate in dose of 1½ grains each given at intervals of one to two weeks. Goodman treated 87 previously untreated latent syphilitic patients with strongly positive Wassermann reactions with 1 grain of mercury salicylate intramuscularly at weekly intervals for courses of from six to eight injections. Tests made immediately after remained strongly positive in 66 per cent. of cases. In only nine was there a reversal to negative and later some of these yielded positive reactions.

Craig[20] has had an unusual experience in the army laboratories. All of 68 cases of syphilis, treated with mercury by mouth for periods ranging from nine months to three years, yielded positive complement reactions, whereas all of these later became Wassermann negative after eight weeks of salvarsan treatment. All of 18 additional cases treated with seven to thirty intramuscular injections of grey oil still gave positive reactions, whereas after salvarsan treatment all became Wassermann negative. Craig does not go so far as to state that the Wassermann test never becomes permanently negative after treatment with mercury, but it has been his experience that it is only in very rare instances that a permanently negative result is obtained from this drug alone, no matter how it is administered or in what dosage. However, he has also made the interesting observation that patients who have been previously well treated with mercury show a higher percentage of negative results after treatment with salvarsan than those who have received no mercurial treatment. Thus, of 75 patients who had received mercurial treatment before receiving salvarsan, 84 per cent. became negative, while of 110 patients who had received no treatment before the administration of salvarsan, only 74.5 per cent. became negative.

Somewhat better results have been reported by White, Hill, Moore and Young[21] with flumerin (disodium salt of hydroxymercurifluorescin) in the treatment of primary and secondary syphilis. In dose of 0.003 gm. per kilogram corresponding to about 0.2 to 0.3 gm. for an average adult, by intravenous injection, flumerin was therapeutically active in the sense that it caused spirochetes to disappear from lesions, brought about the resolution of lesions, and, in about half the cases treated, changed a positive blood Wassermann reaction to negative. In a group of tertiary cases a reduction in the degree of complement fixation, but not always to the negative, was obtained in 9 of 25 cases, while in 16 the reactions remained positive.

My own experience with the new complement-fixation test has been largely confined to the treatment of syphilis with inunctions of mercury and it corresponds very closely with that of Craig, Nelson and Anderson and Goodman referred to above. In other words, cases of chronic syphilis treated over periods ranging from four to ten weeks with daily inunctions have shown only a slight influence upon the complement-fixation reaction, but in no instance reversed the reaction from positive to negative. However, I have reason to believe that periodical courses of mercury by inunction have served to aid in maintaining a state of negative reactions over periods of one to two years; that is to say, have probably eradicated the last vestiges of *Spirochæta pallida* infection or at least have held the infection in check, and thereby prevented sufficient activity of the infection to prevent a reversal of the complement-fixation reactions from negative to positive.

Undoubtedly mercury exerts a therapeutic action in syphilis but mercury influences the complement-fixation reaction much more slowly and effectively than arsphenamin, although a combination of the two yields better results in the treatment of chronic syphilis than either alone.

Influence of Arsphenamin, Neoarsphenamin and Allied Compounds upon Complement Fixation in Syphilis.—Arsphenamin and its allied compounds, as neoarsphenamin and silver arsphenamin, influence complement-fixation reactions in syphilis more profoundly than any other medicaments. Ordinary arsenical compounds, as sodium arsenate, sodium arsenite and

sodium cacodylate, have no influence at all; and pentavalent compounds, like atoxyl, tryparsamid, etc., vary considerably in this regard, as will be discussed shortly in more detail.

As so clearly shown by Detweiler,[22] the influence of these compounds upon complement-fixation in syphilis is best brought out in tests employing varying amounts of serum. The same has been found true with my new test by Schamberg and Klauder,[23] Shivers,[24] Palmer and Gibbs,[25] Kilduffe,[26] Schamberg and Greenbaum[27] and others. These clinicians and serologists have found that, even though the test is very sensitive, negative reactions do occur under treatment, and my own experience has yielded exactly similar results.

In *primary syphilis* the reactions are usually weakly to moderately positive in my new test after the tenth day of the sore. As shown in Chart 1, the reactions are usually reduced to negative after about five doses of neoarsphenamin at intervals of three to four days. Cases of longer duration, with stronger positive reactions before treatment is begun, require more injections and no two cases are ever exactly alike. Sometimes, as shown in Chart 2, a weakly positive reaction is followed by stronger reactions and

Chart 1. Influence of Neo-arsphenamin Upon the Kolmer Complement-fixation Reaction in Primary Syphilis

Reactions.	Serum (c.c.).	Reactions According to Smallest Dose of Serum Yielding a Positive Reaction.												
		Feb. 10	Feb. 12	Feb. 15	Feb. 18	Feb. 21	Feb. 27	Mar. 3	Mar. 10	Apr. 1	May 5	Aug. 10	Dec. 12	
Very strongly positive	0.0025													
Very strongly positive	0.005													
Strongly positive	0.025													
Moderately positive	0.05													
Weakly positive	0.1													
Negative														
Treatment		Before	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	—	—	—	

these in turn by a gradual or sudden reduction to the negative state. I have interpreted such reactions as an indication of ascending spirochetic infection, in spite of one or two doses of neoarsphenamin, followed by destruction of the parasites.

The two charts were plotted according to the smallest doses of serum yielding positive reactions, the method of recording the reactions having been already described in the preceeding chapter. Hardly any two cases ever react just alike, but these charts are fair examples of the average results. It is peculiar how suddenly the reactions will sometimes drop from strongly positive to negative, or jump from negative to positive and back again with the smaller doses of serum. At any rate, practically all cases of syphilis in the primary stage yielding positive reactions can be rendered negative and kept in this state by appropriate treatment with arsphenamin or neoarsphenamin.

Similar results are usually observed in the treatment of *secondary syphilis*. Arsphenamin, however, in dose of 0.6 gm. intravenously usually reverses the complement-fixation reaction from positive to negative more rapidly than neoarsphenamin. As a general rule, positive complement-fixation reactions in cases of secondary syphilis treated with arsphenamin and neoarsphenamin are gradually reversed to negative; but the reduction may not be progressive or orderly, but interrupted by sudden and inexplainable increases of complement-fixation followed by sudden decreases.

In *chronic syphilis*, however, the influence of treatment upon reactions with my new complement-fixation test are much more irregular. In both latent and active cases far more treatment with arsphenamin, neoarsphenamin or sulpharsphenamin is required for reversing the reactions than with the old test which was a modification of Wassermann's method employing three antigens.

CHART 2. INFLUENCE OF NEO-ARSPHENAMIN UPON THE KOLMER COMPLEMENT-FIXATION REACTION IN PRIMARY SYPHILIS

REACTIONS.	Serum (c.c.).	REACTIONS ACCORDING TO SMALLEST DOSE OF SERUM YIELDING A POSITIVE REACTION.												
		Apr. 2	Apr. 6	Apr. 20	Apr. 24	May 10	June 2	Aug. 5	Dec. 10					
Very strongly positive	0.0025													
Very strongly positive	0.005													
Strongly positive	0.025													
Moderately positive	0.05													
Weakly positive	0.1													
Negative														
Treatment		Before	Neo. 0.9	3 doses Neo. 0.9	Neo. 0.9	4 doses Neo. 0.9	3 doses Neo. 0.9	—	—					

No two cases ever appear to react exactly like and practically never in my experience is the reversal from positive to negative orderly and progressive. In chronic latent syphilis especially, the degree of complement fixation is up and down, as shown in Charts 3 and 4, and single negative reactions are of no significance whatsoever. Indeed, a series of two or three consecutive negative reactions in a short space of time is of little significance although negative reactions over a period of one or two years are of much more significance as a criterion of cure.

In both latent and active cases of chronic syphilis, and especially in the former, the complement-fixation reactions are apt to become stronger after one or more injections of arsphenamin or neoarsphenamin, and these effects are attributable, in part at least, to a provocative influence of the drugs upon the parasites. In late congenital syphilis the reactions are particularly difficult to influence and may require two or more years of persistent treatment with neoarsphenamin, mercury and potassium iodid to finally reverse the reactions. In chronic active syphilis, I believe that it is usually easier to

reverse the complement-fixation reactions, with proper medication, that in chronic latent acquired or congenital syphilis.

Silver arsphenamin is regarded by some physicians as somewhat more spirocheticidal than arsphenamin and that positive complement-fixation reactions are reversed to negative more rapidly than with arsphenamin or

Chart 3. Influence of Neo-arsphenamin Upon the Kolmer Complement-fixation Reaction in Active Tertiary Syphilis

Reactions.	Serum (c.c.).	Reactions According to Smallest Dose of Serum Yielding a Positive Reaction.											
		Mar. 5	Mar. 8	Mar. 22	Mar. 29	Apr. 5	Apr. 12	Apr. 19	Apr. 26	May 3	May 10	May 17	June 21
Very strongly positive	0.0025												
Very strongly positive	0.005												
Strongly positive	0.025												
Moderately positive	0.05												
Weakly positive	0.1												
Negative													
Treatment		Before	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	—

Chart 4. Influence of Neo-arsphenamin Upon the Kolmer Complement-fixation Reaction in Chronic Latent Syphilis

Reactions.	Serum (c.c.).	Reactions According to Smallest Dose of Serum Yielding a Positive Reaction.												
		June 21	June 28	July 2	July 9	July 16	July 23	July 26	Aug. 2	Aug. 9	Aug. 20	Aug. 27	Sept. 16	Oct. 17
Very strongly positive	0.0025													
Very strongly positive	0.005													
Strongly positive	0.025													
Moderately positive	0.05													
Weakly positive	0.1													
Negative														
Treatment		Before	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9	Neo. 0.9

neoarsphenamin. I have never used the compound extensively in the treatment of human syphilis and am unable to express an opinion of its influence upon the complement-fixation reaction, although my limited experience has indicated that its effects are about the same as observed with neoarsphenamin.

Sulpharsphenamin likewise influences the complement-fixation reaction, but at this writing I am unable to express a definite opinion as the compound has not yet been employed for a sufficiently long period of time in my practice in the treatment of human syphilis; in a preliminary manner I may state however, that the effects upon my complement fixation reaction have been much less than with arsphenamin and slightly less than in treatment with neoarsphenamin.

Influence of Sodium Cacodylate, Tryparsamid, and Other Arsenical Compounds upon Complement Fixation in Syphilis.—*Sodium cacodylate*, which at one time was regarded as almost equal to arsphenamin in the treatment of syphilis, has practically no influence upon the Wassermann test; as discussed in Chapter XXIII, it is now proven that it has but very slight spirocheticidal activity and is not now generally employed for the treatment of syphilis.

Striking results, however, have been reported by Lorenz, Loevenhart, Bleckwenn and Hodges[28] in the treatment of neurosyphilis with *tryparsamid* alone and in conjunction with mercuric salicylate. The former was injected intravenously in dose of 3 gm. once a week for eight weeks; the salicylate was injected intramuscularly in dose of 1 grain once a week (three days before tryparsamid) for a total of nine injections. After a rest of five to eight weeks this course was repeated, and after another rest was repeated a third time, if the case showed continued activity or was serologically positive. Of 68 cases of paresis, negative reactions were obtained with the sera of 84 per cent. and with the spinal fluids of 30 to 42 per cent. Of 5 cases of taboparesis, negative serum reactions were obtained with 67 to 100 per cent. and negative spinal reactions with 33 to 50 per cent. Of 5 cases of tabes, negative serum reactions were secured with 100 per cent. and negative spinal fluid reactions with 25 to 100 per cent. Of 9 cases of miningovascular syphilis, negative serum reactions were obtained with 75 to 100 per cent. and negative spinal fluid reactions with 37 to 50 per cent. Of 9 cases of generalized syphilis, negative serum reactions were obtained with 42 to 100 per cent. These are truly wonderful results and, if confirmed, will firmly establish the combined treatment of neurosyphilis with tryparsamid and mercury and prove a valuable advance in the treatment of syphilis of the central nervous system.

According to Wright, Kennell and Jussey,[29] *disodium ethyl arsonate* (mon arsone) has an effect upon the Wassermann reaction in syphilis, but as discussed in previous chapters this substance has slight or no trypanocidal and spirocheticidal effects in experimental infections and is not now employed in the treatment of syphilis. Like sodium cacodylate, this compound probably does influence the symptoms of syphilis, but probably more by promoting the absorption of syphilitic exudates than by direct spirocheticidal effects.

Influence of Bismuth upon Complement Fixation in Syphilis.—A review of the literature on the effects of various salts of bismuth, and especially the quadruple salt of potassium and sodium tartrobismuthate, upon the complement-fixation reaction in syphilis, indicates that in this respect bismuth is more active than mercury and less active than arsphenamin and neoarsphenamin. As a general rule, the various bismuth preparations have been injected intramuscularly either in solution or in oily suspension.

Sazerac and Levaditi,[30] who first employed bismuth for the treatment of human syphilis, found that in the primary and secondary stages positive complement-fixation reactions were reversed to negative; likewise, in tertiary syphilis, but very slowly. In more extensive studies by Fournier and Guenot,[31] positive reactions in 35 cases of primary syphilis were reversed to negative in 16; in secondary syphilis none became negative with the first course of treatment, but the majority were reversed to negative with the first course of treatment, but the majority were reversed to negative by a second course. They arrived at no con-

clusions regarding the effects of bismuth upon the Wassermann reaction in tertiary syphilis. Pardo-Castello[32] reports that, of 9 cases of syphilis treated with quinin bismuth iodid, the Wassermann reactions were reversed from positive to negative in 7. Klauder[33] found that sodium and potassium tartrobismuthate in watery solution and in oily suspension, potassium tartrobismuthate in watery solution and bismuth trioxid in olive oil had a favorable influence upon reactions conducted with my new complement-fixation test. The majority of cases of secondary syphilis with strongly positive reactions reacted negatively after the total administration of about 2 gm., while a few required 3 gm. or more before negative reactions were secured. Similar results were observed in cases of primary syphilis. In cases of tertiary syphilis presenting strongly positive reactions, a reversal to negative was observed in some after treatment with each of the three preparations employed. In two cases of early syphilis, however, the sera later yielded positive reactions.

Hopkins[34] reports that a case of primary syphilis with a strongly positive complement-fixation reaction was reversed to negative by six intramuscular injections of potassium and sodium tartrobismuthate in dose of 0.1 to 0.2 gm. at intervals of a week. Similar results were observed in three cases of secondary syphilis. Of 43 cases of latent syphilis with positive Wassermann reactions, 31 of whom had had previous treatment with arsphenamin, mercuric salicylate or both, only one with a strongly positive reaction was reversed to negative by sixteen to twenty-three injections of bismuth and only four with weakly positive reactions were reversed to negative by four to twenty-two injections. A number of patients gave a weakened reaction after their first series of bismuth injections and then had a relapse in spite of subsequent treatment. There were, however, several cases in which considerable treatment with arsphenamin and mercury had exerted no apparent influence, but which showed the first break in their series of strongly positive reactions after taking bismuth. There were also one or two that had relapsed to a weak positive after previous treatment who seemed more definitely influenced by bismuth, and Hopkins surmizes that bismuth may prove useful in the treatment of some of these resistant cases.

Cajal and Spieser[35] have reported that in congenital syphilis the complement-fixation reactions are reversed to negative along with prompt cicatrization of lesions. McCafferty[36] reported that, in 5 cases of primary syphilis, 2 of which gave a positive Wassermann test, the intramuscular injection of 0.2 gm. of potassium and sodium tartro-bismuthate in oil suspension every four days for twelve to sixteen doses resulted in a disappearance of spirochetes in all and negative complement-fixation reactions in 4. In 3 cases of secondary syphilis with strongly positive reactions, two courses of sixteen injections each reversed the reactions to negative. Of 17 cases of tertiary syphilis, 16 of which presented strongly positive reactions, reversal to negative was observed in 3 after one course of twelve injections and in 4 after two courses; in 9 patients receiving one to two courses of injections the complement-fixation reactions were weakened in some but were not reversed to negative.

Practical Value of the Complement-fixation Reaction as a Guide in the Treatment of Syphilis—Lack of Conformity between Symptomatic and Serological Effects.—From the preceding review it is apparent that in a general manner the compounds commonly employed in the treatment of syphilis influence the complement-fixation reaction of the serum in the same ratio as their respective spirocheticidal activities. Arsphenamin is undoubtedly more spirocheticidal than neoarsphenamin and likewise reverses the serological reactions somewhat more promptly; arsphenamin, neoarsphenamin and allied compounds are in turn as a group more spirocheticidal than bismuth, and bismuth more than mercury, and mercury more than potassium iodid, and these relative curative properties are reflected in the same order upon reversal of positive to negative complement-fixation reactions.

It is true that with more sensitive complement-fixation methods the rapidity of reversal is not as prompt and spectacular as in former years when less sensitive methods were employed, but our ideas of the rapidity of cure by arsphenamin and allied compounds have likewise undergone considerable change from clinical developments alone. However, it is common experience to find symptomatic improvement before serological improvement; in other words, courses of treatment sufficient for dissipating the obvious lesions of syphilis may not greatly influence the complement-fixation reactions. Mainly for this reason Kaplan,[37] Wile and Halsey,[38] Oettinger[39] and others do not regard the complement-fixation test as of much practical value in the treatment of syphilis. As previously stated, only another decade or two will

teach us conclusively who is right in this matter. We must watch and determine in the future what proportion of cases develop relapses when treatment is stopped only after securing persistently negative complement-fixation reactions, as compared with those dismissed free of obvious lesions at the time but still serologically positive. My prediction is that the latter group will show a much higher percentage of relapses and I believe it is a serious error to disregard a technically acceptable positive complement-fixation reaction as evidence of existing spirochetic infection, even though the patient has had considerable treatment and is to all intents and purposes recovered clinically as far as this is to be ascertained at the time. All evidence at present indicates that a persistently positive reaction is an indication of continued infection; time may show that is an error, but I do not believe or expect this outcome.

As I shall point out shortly, there is danger, considerably more danger, of placing too much emphasis and reliance upon negative reactions as an index of cure. As shown above, a single negative reaction and even a series of two or three over a period of three or four months may not mean permanent freedom from relapse. There should always be a minimum amount of treatment regardless of negative reactions, because spirochetes may be so reduced in numbers, activity or both to give temporary periods when "reagin" production is not sufficient for yielding positive reactions. All the more reason, therefore, for properly evaluating the positive reaction and accepting it as an evidence of continued spirochetic infection, provided always that it is based upon a technically acceptable method and that the positive reactions persist after suitable periods of rest from specific medication. I repeat as my firm conviction, based upon both clinical and serological experience, that the complement-fixation reaction is a most valuable guide in the treatment of syphilis; that it is usually the last sign of the disease to disappear and the first to reappear if our therapeutic efforts have failed in completely eradicating *Spirochæta pallida* from the tissues and that the goal of our efforts should be to secure persistently negative reactions over years of time with the most specifically sensitive complement-fixation methods available even though this means continuing treatment after the obvious lesions have long since disappeared and even though in some few cases we may be compelled to abandon this goal because of factors preventing or rendering inadvisable the amount of treatment required for attaining such results.

Complement-fixation Reaction as an Index of Relapse in Syphilis.—As just stated, when the complement-fixation reaction is conducted by a specifically sensitive method, it is usually the last sign of syphilis to disappear under treatment; with relatively less sensitive methods, the reactions may be negative in the presence of active lesions and this may occur in rare instances even with sensitive methods, although in such instances errors in technic and especially the use of defective antigen are to be suspected.

Likewise, after the apparent cure of syphilis with a series of negative reactions, the positive reaction is usually the first sign of relapse, the first indication that the spirochetes are still in the tissues and that complete cure has not been effected. Of course, the percentage of relapses depends upon the thoroughness of preceding treatment and figures published by various investigators are of little interest and importance, because methods of treatment have varied so greatly; but percentages, and especially such valuable investigations as those of Craig, have firmly established the principle that recurring positive Wassermann reactions are acceptable as signs of incomplete cure and the resumption of renewed spirochetic activity. As shown by

Craig, relapsing positive reactions bear a relation not only to the stage of the disease but to the kind and thoroughness of treatment.

Just how long a period of time with consistently negative reactions is required before we may feel reasonably sure that relapses will not occur, is a matter largely of personal opinion and will be shortly discussed; but here I hope to make clear that a recurring positive complement-fixation reaction with a technically acceptable method is frequently, and indeed usually, the first sign of relapse and may be present weeks and months before clinical evidences of relapse are apparent. If space permitted, case after case could be here recorded in support of these assertions, but they are so common that doubtless most physicians treating syphilis are sufficiently familiar with them. But we should not wait for clinical evidences before instituting treatment, as this means the loss of much valuable time and the necessity for treating a heavier infection. It is scarcely necessary to repeat again that the method employed should be as sensitive as consistent with specificity and that the tests should be made at intervals over a period of several years for the purpose of detecting the very first evidences of incomplete cure and renewed spirochetic activity.

Complement-fixation Reaction as an Index of the Cure of Syphilis.—And now we arrive at the important question regarding the reliability of persistently negative complement-fixation reactions as evidence of the cure of syphilis.

In the first place, let us get rid of the single negative reaction. Such is absolutely no evidence at all of cure. Frequently and indeed usually, the first negative reaction is followed later by positive reactions. The wise physician will never cease specific medication in syphilis on the evidence of the first negative reaction, even though this occur a year or more after all obvious clinical evidences of the disease have disappeared. The first negative reaction should always be followed by further treatment, but this need not be as intensive as previously employed since a lighter infection remains, as I shall discuss in more detail in later chapters devoted to treatment.

And I hope that I will not tire my readers with again advising the employment of a test yielding reactions as sensitive as possible with practical specificity for syphilis. To be of any value at all the method must yield reactions capable of detecting the first evidences of recurring or relapsing syphilitic infection before such becomes clinically apparent by the production of lesions.

It is commonly stated that a series of negative blood reactions should be observed over a period of not less than a year and preferably two years with a total absence of all clinical signs and symptoms of syphilis, together with a normal spinal fluid, before one may safely express an opinion that complete cure has been obtained. Whether or not this is true I am not able to state. It seems to me that we must suspend judgment and opinion for a sufficient period of time to permit time to tell the story, and time alone can properly evaluate for us our present-day methods and opinions. But I believe that, when these requirements are fulfilled, the results do indicate complete cure, in many cases at least, and especially those cases of syphilis in which active treatment is instituted during the primary and not later than the secondary stages. The longer the disease has been present before treatment is begun the more conservative we should be in accepting a series of negative complement-fixation reactions as a criterion of cure. For example, in cases of primary syphilis I believe that a series of consistently negative reactions with a sensitive method over a period of a year is more acceptable as evidence of cure than two years of negative reactions in case of late secondary or early tertiary syphilis. The reason for this is found in the pathology of syphilis,

since in early cases we have every reason for expecting complete therapeutic success, whereas in the later stages spirochetes firmly entrenched in syphilitic exudates may escape therapeutic destruction, remain in a state of latency and escape detection over long periods of time.

But while we must wait for time to teach us the true value of the consistently negative complement-fixation reaction, something must be done for the present. We should have in mind definite ideas and opinions based upon present-day knowledge for advising our patients and guiding our therapeutic efforts. I am reasonably sure that patients with normal spinal fluids, yielding consistently negative blood reactions with a sensitive method and apparently free of all clinical signs and symptoms of syphilis, need not fear a relapse as long as the blood reactions remain negative. It is possible that in neuro- and cardiovascular syphilis, lesions may gradually develop even though the blood reactions are consistently negative; but if such is suspected in exceptional cases, an examination of the spinal fluid is indicated and if the latter yields negative colloidal gold and complement-fixation reactions, it is highly improbable that active syphilis is present, although I freely admit that latent spirochetes may be present under such conditions. In Chapter LXXIX I have discussed with more detail the curability of syphilis and the criteria of cure, since these subjects require thought and study from several different angles.

Proper Manner of Using the Complement-fixation Test as a Guide in the Treatment of Syphilis and as a Criterion of Cure.—Finally, a few words may be written on this subject. It is commonly stated that blood drawn after the completion of a course of treatment may yield a falsely negative result, whereas after a rest of two to four weeks the true reaction will be obtained. I have never been convinced of the truth of this statement and, indeed, my experience has been just the reverse. That is to say, blood complement-fixation tests conducted during and immediately after a course of treatment are more likely to yield positive reactions than after a period of a rest. And the reasons for this are not difficult to find. In the first place, surviving spirochetes may be active as a result of therapeutic stimulation and thereby furnish maximum amounts of complement-fixing "reagin" during or immediately after a course of treatment. In the second place, even though a course of treatment resulted in complete destruction of all spirochetes, time must be allowed for the elimination of the "reagin" in the blood. Just how long is required for the latter I am not able to state, as it varies of course with the amount to be eliminated; but in syphilitic rabbits cured with single doses of arsphenamin and neoarsphenamin it requires at least three to five weeks to reduce the "reagin" from the ++ level to negative. With human subjects yielding ++++ reactions I believe the matter of elimination to the negative level is a matter of months instead of weeks, but there is no definite way of measuring this more accurately since we can never be sure of cure as we may be in experimental infections of rabbits.

In 1911, Nichols and Craig[40] observed that Wassermann tests, made with blood drawn from strongly positive syphilitics within twenty-four hours after the ingestion of large amounts of beer or whiskey, sometimes reacted negatively and that these falsely negative reactions might continue for as long as three days. Others have apparently confirmed these observations, but I have not had the opportunity of studying the question under properly controlled conditions with human subjects.

My practice in using the complement-fixation test as a guide to treatment and as a criterion of cure is briefly as follows:

1. Blood is never drawn sooner than one week after acute alcoholism.

2. Tests are not made during a course of treatment except as a matter of special study and investigation, or to offer the patient the evidence of serological improvement.

3. Tests are made upon the completion of courses of treatment and again after rest periods.

4. After the last course of treatment, when it is believed that permanent negative blood reactions have been obtained, the tests are done every two months during the following year and every four months during the second year. In cases of chronic syphilis the tests are conducted thereafter, at least twice a year for several more years.

5. The examination of the spinal fluid for the complement-fixation and other examinations is advised and urged routinely in all cases of primary and secondary cases of syphilis after the first course of treatment. In chronic cases and especially those previously treated, these examinations are urged at the same time as the blood test when the patient first comes under observation. Spinal fluid examinations are again advised after the blood complement-fixation tests have yielded negative reactions for at least one year. However, this subject of spinal fluid changes in syphilis, and especially in relation to diagnosis and treatment, is a large and important one and is separately considered in a succeeding chapter.

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CHAPTER XLI

PROVOCATIVE WASSERMANN TEST IN THE DIAGNOSIS OF SYPHILIS AND AS A CRITERION OF CURE WITH SPECIAL REFERENCE TO THE KOLMER COMPLEMENT-FIXATION REACTION

As stated in the preceding chapters, the degree of complement fixation in the Wassermann reaction tends to progressively diminish during the treatment of syphilis with such spirocheticidal compounds as arsphenamin, neoarsphenamin and allied substances as well as with mercury and bismuth. When treatment is begun in the early stages of the disease the decrease in complement fixation is usually progressive until negative reactions are observed. In the treatment of chronic syphilis, however, and especially of latent syphilis with weakly or moderately positive complement-fixation reactions, it is commonly observed that after treatment has been instituted the reactions first become stronger and that the decrease toward negative reactions is more irregular and sometimes broken by sudden and expected negative reactions followed by positive reactions or weakly positive by strongly positive reactions.

Complement fixation in syphilis is regarded as a result and an index of spirochetic activity, although the clinical evidence of a relation between the activity of lesions and the strength of the reactions is frequently lacking; in such cases, however, it is possible that spirochetes are active in physiologically unimportant tissues and while symptoms bear a relation to the location, size and degree of tissue involvement of syphilitic lesions, the quantity of complement-fixing "reagin" bears no relation to location but only to the numbers and degree of spirochetic activity. In "Wassermann-fast" cases of syphilis one is forced to conclude that active spirochetes are still present in the tissues, clinical evidence indicating involvement of the central nervous and cardiovascular systems in such cases; in other words anything increasing the degree of complement fixation in syphilis, that is, a real increase and not due to some technical variation, is to be ascribed primarily to some effect upon the spirochetes and apparently to an increase of their activity, since the Wassermann reaction is acceptable as an index of numbers and activity of these parasites.

As discussed in Chapter XXXV, there is much evidence to show that a variety of factors may provoke a Wassermann reaction in syphilis, but most interest in this chapter is centered upon the question of the influence of antisyphilitic treatment in this relation and whether or not the administration of arsphenamin, neoarsphenamin or mercury may be employed for the purpose of provoking a reaction as a means for the diagnosis of syphilis when the preliminary complement-fixation reaction is negative and as a final means for determining if complete cure has been effected.

Gennerich[1] is generally credited with having first made the observation that the administration of arsphenamin to cases of latent syphilis with negative Wassermann reactions is sometimes followed by a positive reaction and that the procedure may possess practical diagnostic value. He reported twelve cases, but in nearly all only one negative reaction was observed previous to the administration of salvarsan and the resulting "provocative" positive reactions were weak and occurred in from one to fifteen days after injection.

Lerrede[2] and Lier[3] followed some years later with confirmatory evidence and in this country Pease,[4] Craig,[5] Fordyce[6] and others accepted the general proposition that in some cases of latent and uncured syphilis, the administration of salvarsan resulted in the provocation of a positive Wassermann reaction and that the procedure possessed practical value for the diagnosis of chronic latent syphilis and as a test for cure. King[7] and Haller,[8] however, have thought the evidence incomplete and that the positive reactions occurring under such circumstances were due to technical variations in the Wassermann test, as well as to fluctuations in the amount of "reagin" in syphilis regardless of treatment as shown to occur by Boas[9] and Criag[10] and more recently by Browning and Kenneway[11] and Thaysen.[12]

In 1918 Stokes and O'Leary[13] greatly renewed interest in the subject by the study of 103 cases of latent and treated cases. Their plan was to inject intravenously 0.3 to 0.4 gm. arsphenamin or 0.6 gm. neoarsphenamin followed by repeated complement-fixation tests during the ensuing ten days. In 18.4 per cent. of these cases, presumptive but not conclusive evidence of provocative reactions was obtained. In two out of six cases this provocative test was thought of value as indicating unsufficient treatment. In active deep seated visceral, osseous and central nervous system syphilis, it was of less value for diagnosis than in latent syphilis and in cases presenting tertiary lesions of the skin and mucous membranes. Stokes and O'Leary clearly observed and reported, however, that both technical variations and variations in the reagin content of the blood were modifying factors and concluded that the provocative reaction appeared to be of little value in the absence of clinical evidence of the disease and inferior to both clinical judgement and the therapeutic test for the diagnosis of obscure cases.

This report was followed by that of Pollitzer and Spiegel,[14] who administered 0.2 gm. arsphenamin to 150 cases of syphilis presenting negative blood reactions after treatment. Wassermann tests were conducted before and 1, 3, 5, 7, 10 and 15 days after treatment, the series representing a great deal of work, but leading to the conclusion that, on account of various possible errors, no dependence can be placed upon a single positive reaction and that the procedure is useless and misleading.

Klauder,[15] however, had noted evidences of provocative reactions in the spinal fluids of some cases of syphilis in the secondary stage following the intravenous injection of arsphenamin which was in confirmation of the earlier observations of Altman and Dreyfus[16] and Gennerich.[17] In a later study of a larger group of chronic and latent cases of syphilis, Solomon and Klauder[18] observed provocative reactions in the spinal and ventricular fluids of some cases of chronic syphilis after the intravenous and intraspinous injections of arsphenamin consisting of an increase of cells and protein as well as positive complement-fixation reactions. In none of these cases, however, were provocative blood reactions observed, but it was believed that the provocative spinal fluid changes were occasionally of value in aiding the diagnosis of obscure cases.

In the meantime Uddgren[19] published observations tending to show that the intramuscular injection of sterile milk in doses of 5 to 10 c.c. provoked positive Wassermann reactions in some cases of latent syphilis, probably due to non-specific stimulation of latent foci of spirochetes and analogous in this respect to the stimulation of a latent case of syphilis by trauma caused by a bullet and the injection of tetanus antitoxin, occurring in the practice of Dr. Pelouze, of Philadelphia, and recorded by Klauder.[20]

Finally, O'Leary[21] in a second study of 285 cases in the Mayo Clinic, observed apparently true provocative reactions in 8 per cent. of cases following the intravenous injection of 0.3 gm. of arsphenamin with a series of seven complement-fixation tests thereafter, at intervals of twenty-four hours. Mention may also be made of the investigation of Frei[22] who commonly observed an apparent increase of spirochetes in the chancres of a number of cases following the intravenous injection of salvarsan and interpreted as indicating the direct spirocheticidal activity of this substance, the study having been undertaken to throw additional light upon the mechanism of the action of salvarsan in syphilis.

This brief review of the literature indicates, therefore, a considerable difference of opinion regarding the question of whether the administration of arsphenamin or neoarsphenamin actually does provoke positive complement-fixation reactions in latent and uncured syphilis or whether such apparent reactions are due rather to technical variations and fluctuations of the Wassermann "reagin" in the blood independent of the injection; also regarding the practical or clinical value of the provocative complement-fixation reaction in the diagnosis of obscure cases of syphilis and as a test of cure, granting that true provocative reactions occur.

During the past two years, I have been much interested in a study of the subject with my new complement-fixation test, believing that its quantitative character and precautions against technical errors may yield additional

information. The results of these studies are briefly summarized in the balance of this chapter and likewise my general conclusions upon the whole subject of the provocative Wassermann reaction based thereon.

Variations in the Degree of the Complement-fixation Reaction Due to Technic.—Unquestionably minor degrees of variation in the degree of complement-fixation are due to technical factors over which there is no adequate control under routine conditions. These variations are largely due to variations in the complement serum and to lesser extent to variations in the anticomplementary activity of human sera providing the same antigen is employed in all tests. If, however, different antigens are employed in tests of the serum of the same individual at different times over a period of observation, a new and important additional factor of variation is introduced.

With my new method however, the degree of variation presumably due to technic is very slight and usually elicited only in the smaller doses of serum. I have, for example, frequently taken specimens of blood each day for eight to ten days from cases of tertiary and latent syphilis and conducted tests each day and again repeated all of the tests on the same day with the same complement, same antigen, etc., with specimens of each serum carried over at o°C. The following results observed with one case of chronic untreated latent syphilis is representative of the series:

Daily tests with specimens of serum yielded the following reactions:

Serum C.C.	Days									
	1	2	3	4	5	6	7	8	9	10
0.1	4*	4	4	4	4	4	4	4	4	4
0.05	4	4	4	4	4	3	4	4	4	3
0.025	2	1	2	2	1	2	1	1	2	1
0.005	–	–	–	–	–	–	–	–	–	–
0.0025	–	–	–	–	–	–	–	–	–	–

*4 = ++++; 3 = +++; 2 = ++; 1 = +.

The results of tests conducted with all ten specimens of serum on the same day yielded the following reactions:

Serum C.C.	Days									
	1	2	3	4	5	6	7	8	9	10
0.1	4	4	4	4	4	4	4	4	4	4
0.05	4	4	4	4	4	4	4	4	4	4
0.025	4	4	2	2	2	1	1	1	2	1
0.005	–	–	–	–	–	–	–	–	–	–
0.0025	–	–	–	–	–	–	–	–	–	–

It will be observed, therefore, that the reactions were slightly stronger in this set of tests with the first and second day sera, due, presumably, to the acquisition of slightly increased anticomplementary properties of these sera even though the serum controls were completely hemolysed.

It will be noted, however, that such uniform results were observed only in selected chronic untreated latent cases of syphilis, which are the only ones suitable for tests of this kind in order to reduce to a minimum variations in the degree of complement-fixation due to possible fluctuations in the amounts of "reagin" in the blood. In such cases the new test has yielded remarkably uniform reactions, the same antigen being used in the same dosage in all tests. As previously stated, the fluctuations were observed only in the higher dilutions of serum and never in the larger doses as 0.1 and 0.05 c.c. of cases yielding strongly positive reactions.

Variations in the Degree of Complement-fixation Reactions Due to Fluctuations in Antibody.—My studies in this subject have been confined to untreated chronic cases of syphilis, as there is an abundance of evidence to show that in untreated acute syphilis in the primary and secondary stages the complement-fixation reactions fluctuate, but with a tendency to become stronger and stronger. The general result has been to show that, in *tertiary syphilis with active lesions*, a marked variation in the degree of complement-fixation occurs which may be reasonably ascribed in most part to fluctuations in the "reagin" content of the serum.

For example, in a case presenting an ulcerating gumma of the nose, the reactions conducted with ten specimens of serum collected over a period of seventeen days but all tested at the same time in order to reduce to a minimum fluctuations due to technic, were as follow:

Serum C.C.	Days									
	1	2	3	5	6	9	11	12	14	17
0.1	4	4	4	4	4	4	4	4	4	4
0.05	4	4	3	4	4	2	3	4	4	4
0.025	4	4	–	4	3	–	–	1	2	1
0.005	2	1	–	–	–	–	–	–	–	–
0.0025	–	–	–	–	–	–	–	–	–	–

It will be observed that the fluctuations occurred principally with the smaller doses of serum and I have not yet observed a case of untreated syphilis that yielded a strongly positive reaction one day and a negative reaction the following day, although with weakly reacting sera this was sometimes observed, as shown in the following results of tests conducted on one day with six specimens of sera collected over a period of eleven days from a case of untreated chronic syphilis showing beginning optic atrophy:

Serum C.C.	Days					
	1	2	5	9	10	11
0.1	2	2	–	2	1	2
0.05	–	–	–	–	–	–
0.025	–	–	–	–	–	–
0.005	–	–	–	–	–	–
0.0025	–	–	–	–	–	–

In tests conducted over longer periods of time the fluctuations in degree of complement-fixation were more marked; in these cases, however, the tests were conducted with each separate specimen of serum about forty-eight hours after bleeding and for this reason introduced the added technical factors of variation ascribed to the use of different complement sera, as well as, possibly, to minor and undetectable changes in the antigenic activity of the extract employed as antigen. The results of one such case presenting chronic syphilitic aortitis and myocarditis but in whom treatment could not be undertaken at the time owing to deficient urinary elimination due to chronic nephritis, were as follows over a period of 35 days:

Serum C.C.	Days						
	1	5	8	10	14	21	35
0.1	4	4	4	4	4	4	4
0.05	4	3	4	1	4	4	4
0.025	–	–	1	–	2	–	–
0.005	–	–	–	–	–	–	–
0.0025	–	–	–	–	–	–	–

In *chronic latent syphilis* the fluctuations are less marked, my tests having been conducted with the sera of untreated syphilitic multiparous women presenting no other evidences of syphilis than syphilitic progeny and positive complement-fixation reactions. In one such case, examined about two years after the last pregnancy, the reactions were as follows, the tests being conducted separately over a period of 42 days and therefore, including technical variations:

Serum C.C.	Days								
	1	2	5	9	17	21	28	36	42
0.1	3	4	3	1	1	2	3	4	3
0.05	1	1	2	–	–	1	–	2	–
0.025	–	–	–	–	–	–	–	–	–
0.005	–	–	–	–	–	–	–	–	–
0.0025	–	–	–	–	–	–	–	–	–

I believe, therefore, that it may be accepted without reserve that the Wassermann reaction varies in the degree of complement fixation due to fluctuations of the "reagin" content of the sera. Undoubtedly a portion of this variation is due to the technical factors mentioned above but allowing for these we cannot escape the conclusion that the amount of "Wassermann substance" or "reagin" likewise fluctuates and is responsible for varying degrees of complement fixation. This fluctuation may not be detected with daily specimens of blood over a period of less than a week, but is well defined with tests conducted over a period of several weeks or months. This is especially true in chronic syphilis presenting active lesions; it is less apparent in chronic latent syphilis. In both groups, however, variation in complement fixation may bear no relation to the clinical condition of the patient. That is to say, the reaction may be strongly positive at one time and weaker at another without coincident clinical manifestations of exacerbation or regression of the disease or a particular lesion. In my experience, the new method employed in these studies has shown less variation than my former method employing three antigens and, in the latter, cholesterolized antigens showed less variation than antigens of aceton insoluble lipoids and plain alcoholic extracts of syphilitic liver or beef heart. These differences are ascribed to the use of a new antigen of superior qualities in the new method, together with a combined primary incubation of 18 hours at 6 to 8°C. followed by ten minutes in a water-bath at 38°C., as well as to the method employed of preparing and titrating the complement serum and to other technical factors of lesser importance.

Variations in the Degree of Complement-fixation Reactions Due to Treatment.—These have been previously referred to in Chapter XL. In the treatment of acute syphilis embracing the primary and secondary stages, the variations in degree of complement fixation are not extreme, but in the treatment of chronic syphilis both active and latent, extreme variations are sometimes encountered and especially after treatment has been underway for several months. One commonly observes that, during the first few weeks of treatment of chronic syphilis the reactions become stronger followed by a gradual decrease in complement fixation to negative reactions, but with, frequent exacerbations; in the co-called "Wassermann-fast" cases, however much less variation in the degrees of complement fixation is observed.

Diagnostic Value of the Provocative Complement-fixation Reaction in Syphilis.—I believe that it must be admitted that spirocheticidal drugs, and especially arsphenamin and neoarsphenamin, are capable in subcurative doses of disturbing foci of spirochetic infection with an exacerbation of their

activities. The Jarisch-Herxheimer reaction is commonly believed to be a phenomenon of this kind and the provocative Wassermann reactions have been considered of this same general nature although Stokes and O'Leary have observed no constant relation between clinical exacerbation (Jarisch-Herxheimer reactions) and positive provocative complement-fixation reactions.

But my experience coincides with that of Stokes and O'Leary that provocative reactions are only likely to occur in the presence of clinical evidences of syphilis and not in latent syphilis. In other words, the provocative complement-fixation reaction requires that there be present in the tissues a minimal number of spirochetes to be provoked into activity for the production of the complement fixing "reagin." In not a few cases of chronic latent syphilis and especially of treated cases, this condition may not be present. Furthermore, the spirochetes must be readily accessible to the arsphenamin or neoarsphenamin and while at first thought this may not appear to be of much importance, yet the provocative test is usually conducted by the administration of single doses and in chronic syphilis it may well be that a single dose of arsphenamin or neoarsphenamin is eliminated before appreciable spirochetic effects are brought about.

I believe, therefore, that the provocative Wassermann reaction by the administration of arsphenamin or neoarsphenamin is a demonstrated fact, but its practical diagnostic value is an entirely different matter. In chronic syphilis with lesions the injection of a single dose of arsphenamin or neoarsphenamin has in my experience undoubtedly increased the strength of the new complement-fixation reaction, but in latent syphilis with two to five preliminary negative reactions, I have not yet observed convincing evidence of a provocative reaction with blood serum after single doses of arsphenamin or neoarsphenamin and have had no experience with spinal fluids in this particular regard.

In other words, if a patient presents a lesion that may be syphilitic but with a negative-complement fixation reaction, I believe that it is decidedly worth while to administer arsphenamin or neoarsphenamin after the plan described below; in such cases positive serum reactions have been provoked and at the same time a therapeutic test is made which may be even more valuable for diagnosis.

But in latent syphilis without manifest lesions I do not regard the test with single injections of drug as worth while despite the fact that one may occasionally elicit very weakly positive reactions; in some such cases a series of doses of neoarsphenamin has yielded more conclusive evidence and if one is reasonably assured that the individual is syphilitic, as may be true with the mothers of syphilitic children, no harm is done by the administration of several doses; otherwise I agree with Shepardson[23] that just as much or more can be accomplished for diagnosis by conducting occasional complement-fixation reactions by a sensitive method without resorting to the provocative procedure.

Sometimes the administration of a dose or two of arsphenamin or neoarsphenamin is followed by clinical exacerbations of syphilis and I believe that the physician should always keep such individuals under observation for several weeks at least and be prepared to proceed at once with active treatment if such occurs. In latent syphilis of the aged or of individuals with nephritis or some such condition rendering inadvisable the institution of active treatment, my advice is not to employ provocative injections but to leave alone what is good enough at least for the time.

Value of the Provocative Complement-fixation Reaction as an Index of the Cure of Syphilis.—While the provocative procedure may be of aid in the diagnosis of obscure lesions of syphilis and especially if a series of injections of neoarsphenamin are given, I have observed no value whatsoever to provocative tests with *single* doses of arsphenamin or neoarsphenamin as a criterion of cure. Here again, however, the administration of three doses of neoarsphenamin has sometimes resulted in provoking weakly positive reactions with the sera of individuals who had finished courses of active treatment with repeated negative reactions during periods of rest. For this reason, I always conduct frequent tests during the last one or two courses of treatment given routinely after the complement-fixation reaction has first become negative, because if positive reactions are observed it is taken as an indication for a more thorough course of treatment than would otherwise be indicated.

After the complement-fixation test has yielded a series of negative reactions, accompanied by clinical evidences of cure insofar as these may be ascertained, I believe that complement-fixation tests at periodical intervals of three or four months for the ensuing two years are sufficient and that provocative tests employing single doses of arsphenamin or neoarsphenamin as usually conducted are useless and may give a false sense of security.

Author's Method for Conducting the Provocative Complement-fixation Reaction.—If the provocative procedure is employed as an aid in the diagnosis of an active lesion of obscure origin, of chronic latent syphilis or possibly as a test of cure, I believe that at least two or three doses of neoarsphenamin should be given. In the majority of cases in which this test is employed, there are usually good reasons for suspecting syphilis and at any rate the injection of neoarsphenamin cannot be said to be dangerous or harmful. Just how long one should wait after the injections before drawing blood for the tests cannot be stated as it involves the question of how rapidly the "reagin" is produced. Under ordinary circumstances the procedure is apt to be too expensive for many individuals and for this reason the number of tests should be reduced to a minimum. My own practice is as follows, the test being applied only to occasional and exceptional cases and especially those presenting clinical manifestations or a history suspicious of syphilis:

(*a*) The intravenous injection of 0.6 gm. neoarsphenamin dissolved in 20 c.c. of sterile distilled water.

(*b*) Blood withdrawn 48 hours later and the complement fixation test conducted; if the reaction is negative:

(*c*) A second intravenous injection of 0.6 gm. neoarsphenamin five days after the first.

(*d*) Blood withdrawn 48 hours later and the complement-fixation test conducted.

Sometimes this is sufficient if a positive reaction is observed, but under these circumstances the result is checked up by a third test. If, however, the reaction is negative, a third injection of neoarsphenamin is given followed by a blood test forty-eight hours later. If this test yields a negative reaction a final blood test is taken five days later or from seven to ten days after the last injection. All of the complement-fixation tests are conducted according to the technic of my new quantitive test employing varying amounts of serum, as this test has been demonstrated to possess a very high degree of sensitiveness combined with specificity for syphilis. It is important to employ a sensitive test for results; tests conducted in the usual manner with primary periods of incubation of one hour in water bath or incubator are not nearly as satisfactory because lacking in sensitiveness and especially if plain

alcoholic extracts of syphilitic liver, beef or guinea-pig heart are employed as antigens.

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CHAPTER XLII

CEREBROSPINAL FLUID CHANGES IN RELATION TO THE DIAGNOSIS AND TREATMENT OF SYPHILIS

The detection of invasion and infection of the organs of the central nervous system with *Spirochæta pallida* is greatly aided by the examination of the cerebrospinal fluid. The positive blood complement-fixation reaction indicates a general invasion and infection of the body, but in early syphilis may afford no evidence of the involvement of any particular organ or tissue, and clinical and roentgenological evidences of infection are not usually to be elicited until the disease has been well established with the production of chronic lesions. However, infection of the meninges, and even of the substance of the brain and cord, may be detected by appropriate examinations of the spinal fluid during the first stage of infection of these tissues. Indeed, it is probable that in some cases, at least, the spinal fluid changes in the late primary and secondary stages of syphilis are due merely to invasion of these organs with *Spirochæta pallida* during the acute spirochetemic stage of the disease, without infection of their tissues, since permanent localization and survival of the parasites may be prevented by local factors of resistance.

Spirochetic Invasion versus Infection of the Central Nervous System.—In other words, a distinction should be made between the invasion and the infection of organs with *Spirochæta pallida*, just as we do in bacterial diseases. In the former, the parasites gain access to the tissues and may even produce temporary disturbances, but are destroyed or dislodged by natural immunological agencies before tissue changes occur and infection established. Studies of the cerebrospinal fluid, which I shall review shortly, have indicated

that, during the acute stages of syphilis and particularly the late primary and secondary stages, evidences of spirochetic invasion of the meninges and other tissues of the central nervous system are to be found in at least 30 to 40 per cent. of cases; treatment, and especially the active and continuous administration of arsphenamin and neoarsphenamin for six or more doses, reduces the incidence of these changes to 15 to 25 per cent., but even in some untreated cases of syphilis these changes disappear spontaneously, indicating that while the tissues may be invaded by *Spirochæta pallida*, infection does not necessarily follow. It is doubtful if the administration of pills of protiodid of mercury in the old method of treating syphilis resulted in exerting sufficient spirocheticidal activity to actually prevent infection of the tissues of the central nervous system after invasion had occurred; yet the incidence of syphilis of these organs under this old plan was probably not over 10 per cent. and especially in the negro, indicating that natural resistance of these tissues may, in some cases, at least, prevent infection even though invasion has occurred. However, when invasion has occurred to the extent of producing pleocytosis and an increase of proteins in the cerebrospinal fluid, the danger of infection is imminent unless the parasites are promptly destroyed by chemotherapeutic measures. Some physicians believe, however, that active medication may actually aid in producing infection of these tissues at this time by breaking down the natural protective agencies, by preventing the production of antibodies or producing breaks in the capillaries, but I shall discuss these hypothetical questions below. The point I wish to make here is that, in my opinion, the examination of the spinal fluid has been brought up to such a state of perfection at present, that invasion of the central nervous system with spirochetes may be detected in the majority of cases of early syphilis, but that the evidences of this invasion do not necessarily mean that infection will occur with the development of lesions producing clinical neurosyphilis and especially if syphilis is properly treated during its early stages.

There is no reason to doubt that the tissues of the central nervous system are invaded with spirochetes in all cases of syphilis in which spirochetemia develops. The same is probably true of all other vascularized tissues of the body. It is interesting, however, that spirochetic invasion of the brain, cord and meninges produces spinal fluid changes in the majority of cases and that the spirochetes themselves may be actually found in the fluid even in the primary and secondary stages of syphilis. But even in those cases whose spinal fluid shows no changes, spirochetic invasion cannot be excluded; indeed, spirochetes have been found in the spinal fluids of such by rabbit inoculation. I see no reason, therefore, for spinal puncture and the examination of spinal fluid for evidences of neural invasion and infection until sufficient treatment has been given to sterilize the blood; after this time, however, in practice after the first course of treatment, examination of the spinal fluid is indicated for the detection of those cases in whom the spirochetes have succeeded in remaining in the tissues and escaping destruction.

Just what factors determine whether or not infection will occur after invasion has taken place—that is, whether or not spirochetes will lodge in the tissues and produce lesions after being brought to them by the blood—cannot be stated, just as we are unable to explain at present why spirochetes locate mainly in the myocardium and aorta of some cases, in the liver in others, etc. It may be that some strains have a specific tissue affinity for certain organs, that localization may be determined by preceding tissue injury lowering local resistance or favoring blood stasis, but these subjects have been already discussed in Chapter XXXVII. Very probably the

natural resistance of different organs varies considerably in resistance to infection after being invaded by *Spirochæta pallida* and that the tissues are not equally protected by the general reaction which occurs during the early stages of syphilitic infection; I believe such factors, together with preceding tissue injuries reducing local resistance or favoring blood stasis, are the important determining factors governing infection of the central nervous system as well as other organs of the body. At any rate, in cases of neurosyphilis, spirochetic infection may be found in other parts of the body as shown by Fordyce,[1] Stokes,[2] Wile,[3] Frisch[4] and others; aortitis, myocarditis and even skin and periosteal lesions are frequently associated, all due to the same strain and constituting very positive evidence against the acceptance of the theory of specific neurotropism of strains of *Spirochæta pallida*. Treatment of syphilis at the time of initial neural invasion is, however, of great importance, since it would appear from the investigations of Wile, Moore, Keidel, Stokes and others shortly to be reviewed, that prompt destruction of the spirochetes at the time of invasion reduces the chances of infection of these tissues just as it does of other organs. In my opinion there is no evidence at all to indicate that arsphenamin or neoarsphenamin, administered in a proper manner, results in actually aiding infection of the central nervous system (neurofixation) during the early stages of syphilis, as claimed by Mallein, Tzanck and Kesmorgant[5] and others, by reducing antibody production or producing minute injuries of the capillaries.

Spirochæta Pallida versus Spirochetic Toxins in the Etiology of Neurosyphilis.—Prior to 1913, when Noguchi and Moore[6] discovered *Spirochæta pallida* in the brains of twelve cases of paresis, the majority of neurologists were of the opinion that syphilis played some etiological rôle in at least some cases of paresis and tabes, but the relationship of *Spirochæta pallida* to these diseases was not clearly established and they were commonly designated as parasyphilitic or metasyphilitic diseases. This discovery was quickly confirmed by Levaditi, Marie and Bankowski,[7] who found *Spirochæta pallida* in the fresh substance of eight in a series of nine paretic brains, and since then confirmatory evidence has been supplied by the investigations of Nichols and Hough,[8] Graves,[9] Wile[10] and others. Noguchi also found *Spirochæta pallida* in the spinal cords of a few tabetics; Reasoner[11] in the brain of an early case of cerebral syphilis; Kiely[12] in the cerebral cortex of congenital syphilis, etc. Except in paresis, however, the number of spirochetes to be found in the meninges, spinal cord, etc., are very small and their demonstration usually requires prolonged search of a large number of stained sections.

While Uhlenhuth and Mulzer[13] failed to demonstrate *Spirochæta pallida* in the spinal fluids of cases of paresis and tabes by inoculation into rabbits, they were later successful with the fluid of a case of early cerebrospinal syphilis, and since then Hoffmann, Nichols and Hough, Steiner,[14] Volk,[15] Artz and Kerl,[16] Fruhwald and Zaloziecki[17] and others have reported positive results. In this country, Wile[18] has made particularly valuable studies reporting successful rabbit inoculations with as high as 62½ per cent. of spinal fluids from cases of early syphilis, of tabes and of paresis. More recently Wile and Kirchner[19] have demonstrated spirochetes in the spinal fluid of cases of syphilis in the primary and secondary stages of the disease, as well as in cases of chronic neurosyphilis, by sections of coagula produced by a modification of the Alzheimer method and stained by the Warthin-Starry silver agar method. Just what governs the access of spirochetes to the spinal fluid cannot be stated, but probably this is determined by the degree of spirochetemia and the migration of parasites from the perivascular lymph spaces. If perivascular invasion is extensive it is readily understood how spirochetes may be washed by a more or less mechanical manner into the secretions of the choroid plexes or by extravasation of plasma from the vessels of the meninges and particularly of the pia into the spinal fluid. This is particularly true if *Spirochæta pallida* excites some inflammatory changes in these tissues with serous exudation. Investigations of this kind indicate, therefore, that *Spirochæta pallida*, rather than hypothetical toxins produced in some other focus of syphilitic infection and carried to the central nervous system, is directly and primarily responsible for the lesions.

With the syphilitic psychoses the evidence is less conclusive, but data at hand indicate that *Spirochæta pallida* produces but little diffusible toxin and it is highly probable that the true psychoses of syphilis are likewise due to the actual presence of spirochetes in the brain rather than to the influence of toxins or toxic substances produced by spirochetes or the disintegration of syphilitic exudates elsewhere in the body and transported to the central nervous system by the blood and lymphatic systems.

Anatomical and Clinical Classification of Neurosyphilis.—No subject in syphilis is more perplexing from the standpoint of classification of lesions and symptom-complexes than syphilis of the nervous system. For this reason the term "neurosyphilis" has come into general use for designating a wide variety of lesions and diseases of the nervous system and particularly of the central organs due to syphilitic infection. For example, the term includes lesions of the peripheral nervous system, embracing the cranial and peripheral nerves, their ganglia and end organs, as well as the sympathetic nerves, their ganglia and terminal arborizations; likewise lesions of the brain, spinal cord and meninges, constituting "central neurosyphilis." From the pathological standpoint we hear of vascular neurosyphilis, parenchymatous neurosyphilis, diffuse neurosyphilis, gummatous neurosyphilis, etc. From the clinical standpoint the terms are even more numerous and perplexing, the commoner being paresis, paretic neurosyphilis or general paralysis of the insane; tabes dorsalis, or locomotor ataxia, taboparesis, cerebrospinal syphilis, cerebral syphilis, cerebrospinal sclerosis, syphilitic arteriosclerosis and thrombosis, etc., etc., as well as many more terms to include the mental or psychic disturbances as syphilitic paranoia, tabetic psychosis, syphilitic neurasthenia, "paresis sine paresi," etc., etc.

In other words, *Spirochæta pallida* may infect any portion of the nervous system and depending upon the anatomical location of the lesions we have a pathological classification; according to the signs and symptoms produced we have a clinical classification.

I would not have presumed to touch upon this intricate matter of classification of neurosyphilis, were it not necessary to do so for the adequate discussion of cerebrospinal fluid changes in syphilis and especially in relation to chemotherapy. To discuss the whole subject under the general term "neurosyphilis" is not sufficient because an adequate knowledge of the fundamental pathological changes is required for the proper evaluation of the results of examination of the spinal fluid as a means of diagnosis, as a guide in treatment and an index of cure; indeed, such knowledge is likewise indispensable for the proper evaluation of therapeutic measures and in relation to prognosis. Some physicians cannot understand why the Wassermann test with spinal fluid should yield a negative reaction in some cases of neurosyphilis or why the cells are almost within normal limits in some cases and increased in others. In no other system may there be such appalling discrepancies between clinical and laboratory findings as in neurosyphilis and yet a few minutes of thought on the subject is sufficient for an understanding of the chief reason, namely, in no other system may size and location of syphilitic lesions so profoundly influence the clinical signs and symptoms.

I do not believe, however, that it is necessary for the general practitioner to have in mind the elaborate classifications of the neuropathologist or the clinical neurologist and psychiatrist. It is sufficient to remember that *Spirochæta pallida* may locate primarily in the meninges, in the substance of the brain or cord, in or about the blood-vessels or in all three at the same time, producing respectively, meningeal, parenchymatous, vascular and diffuse neurosyphilis. The lesions may be general or local and are usually multiple; they are seldom of pure types. For example, meningeal syphilis may involve the parenchyma and parenchymatous syphilis almost always involves the overlying pia mater. It is for this reason that systematists have been compelled to adopt rather lengthy classifications. My own working classification has been largely based upon the excellent classifications of Southard and Solomon and is here given for any aid it may be to others:

Anatomical	Clinical
Meningeal neurosyphilis (changes principally in the meninges)	1. Acute and chronic cerebral or cerebrospinal syphilitic meningitis (so-called cerebrospinal syphilis)
	2. Chronic localized syphilitic meningitis which may result in: Gumma of meninges; Cranial nerve palsies; Bulbar palsy; Neuritis and muscular atrophy; Transverse myelitis; Epilepsy
Vascular neurosyphilis (changes principally in the blood-vessels)	1. General syphilitic arteriosclerosis
	2. Localized syphilitic arteriosclerosis which may result in: Apoplexy; Aneurysm; Cerebral thrombosis and epilepsy
Parenchymatous neurosyphilis (changes principally in the substance of brain or cord)	1. General syphilitic sclerosis of brain and cord (cerebrospinal sclerosis) with gummas
	2. More or less localized syphilitic sclerosis with nerve cell destruction: Paranoia; Chorea; Epilepsy; Muscular atrophy
Diffuse neurosyphilis (mixtures of meningeal, vascular and parenchymatous)	1. Paresis, congenital ("juvenile paresis") and acquired ("general paresis;" "dementia paralytica," "softening of the brain")
	2. Tabes dorsalis and tabetic psychosis
	3. Taboparesis
	4. Erb's syphilitic spastic spinal palsy
	5. Some cases of cerebrospinal syphilis

Spinal fluid examinations in conjunction with the blood Wassermann test usually furnish sufficient data for establishing the general diagnosis of syphilis of the central nervous system, but for purposes of differential diagnosis are of aid only when the results are interpreted with the clinical signs and symptoms. In other words, there are no pathognomonic signs of any one syphilitic disease of the nervous system to be found in the spinal fluid; probably the colloidal gold reaction in paresis most nearly approaches this state, but in some other non paretic syphilitic diseases a similar gold curve may be produced. In many cases, probably the majority, a sufficiently accurate diagnosis of neurosyphilis with determination of the location of the lesion or lesions may be made by ordinary neurological examination embracing the papillary reactions, various reflexes, gait, station, etc., together with spinal fluid examination; in others, however, prolonged experience and expert knowledge are required, and especially in the diagnosis of the psychoses and their differentiation from organic diseases, as manic depressive psychosis and dementia precox from paresis, etc., etc.

Pathology of Neurosyphilis in Relation to Spinal Fluid Changes.—As previously stated, the lesions of neurosyphilis are due to the presence of *Spirochæta pallida* rather than to some hypothetical toxin produced elsewhere in the body and transported to these tissues. The parasites are brought to the tissues of the central nervous system by the blood and there is no reason to doubt but that this occurs in all cases of untreated syphilis passing through the primary and secondary stages of the disease. In those cases in whom this initial invasion is followed by infection or those whose tissues are invaded and infected during temporary periods of spirochetemia in the late stages of syphilis, which I believe may occur, the spirochetes lodge in the perivascular tissues. From this time on the fate of the individual depends upon the numbers, invasiveness and virulence of the spirochetes. If the parasites are but feebly invasive and virulent, they may progress no further and vascular lesions of sclerosis result; if the parasites are highly invasive and virulent, they may migrate with the involvement of surround-

ing tissues producing more diffuse lesions of the meninges or parenchymatous structures.

The resulting lesions are essentially similar in their histological characteristics. *Spirochæta pallida* exerts positive chemotaxis upon small lymphocytes and the areas of syphilitic infection are infiltrated and surrounded with these cells. Plasma cells are likewise found. Congestion with serous exudation occurs as in other inflammations, and as a result both cells and serous inflammatory fluids may find their way into the sipinal fluid, producing an increase of cells (pleocytosis) and proteins. Hence, the degree of these changes varies with the acuteness of infection, being most marked in the early stages of the process because fibrous tissue is laid down later in and about the lesions as in all chronic inflammations which tends to reduce cellular and serous exudation. As the result of poisonous products of the spirochetes plus a reduction in nutrition due to endarteritis and lymphocytic crowding, the tissue cells of the infected areas may be destroyed with consequent impairment or loss of function. Doubtless, the products of this disintegrative process reach the spinal fluid, producing other changes in the organic and inorganic constituents which are detected only by elaborate chemical analyses.

It is surprising, however, how much damage may be noted upon histological examination of the tissues with so few spirochetes to be found. Doubtless, however, spirochetes tend to die and undergo autolysis during the fibrotic stage of the lesions and the scarcity of their numbers in chronic lesions of the nervous system (paresis excepted) is analogous to conditions in syphilitic lesions elsewhere in the body. Doubtless more spirochetes would be found if it were possible to examine the early lesions and especially if the tissues could be fixed within an hour or two after death. However, one never ceases to wonder at the amount of tissue change, and especially the degree of fibrosis, that may be brought about by what must be relatively few parasites. Of course, in the central nervous system, extensive tract degeneration and fibrosis may be due to a small lesion involving the tract at one point with descending or ascending degeneration from this point. For this reason the examination of numerous serial sections of tissue may be required, before foci of infection are discovered.

Almost needless to state, the degree and, to a lesser extent, the kind, of spinal fluid changes in neurosyphilis vary according to the situation and size of the lesions. The cells and proteins are especially increased when the meninges are involved and the amount of increase is almost an index of the degree of meningeal involvement; the colloidal gold and complement-fixation reactions, however, are more influenced by the degree of parenchymatous involvement.

Nowhere else in the body may there be such a lack of conformity between signs and symptoms of disease and laboratory findings as in neurosyphilis. For example, in tabes dorsalis and Erb's spastic paraplegia the initial sclerosis of the posterior nerve roots followed by sclerosis of the posterior columns may produce the most marked signs of disease and yet the lesions are so small and of such a kind that the cerebrospinal fluid changes are apt to be slight and, indeed, may not be appreciable at all in some cases.

The same is true in some other syphilitic lesions of the central nervous system. For example, a small patch of syphilitic infection involving a cranial nerve may bring about a palsy, producing marked signs and symptoms, and yet the lesion may, on account of its small size or situation, produce no demonstrable changes in the spinal fluid. A gumma of the meninges may do a great deal of mischief with muscular atrophy and other changes

due to involvement of spinal tracts and nerves, and yet the actual degree of pathological changes may be so slight that abnormalities in the spinal fluid cannot be elicited; even extensive tract degeneration with fibrosis (gliosis) may produce no increase of cells or of protein, although other changes in the chemistry of the spinal fluid may occur, which, however, cannot be detected by simple routine examinations.

Therefore, *it should never be overlooked by clinicians that negative spinal fluid findings do not exclude the possible syphilitic nature of a lesion or a disease;* Solomon and Klauder[20] have especially directed attention to the possibility of negative spinal fluid findings in some cases of neurosyphilis and particularly in vascular lesions, as well as in tabes, cerebral gumma, cerebral nerve palsies, syphilitic epilepsy and the psychoses, as syphilitic paranoia and syphilitic dementia. Just as I have emphasized in the preceding chapter that a negative blood-complement-fixation reaction does not exclude syphilis, the same may be said of the spinal fluid. However, owing to the fact that the spinal fluid may be used in larger amounts than serum, the complement-fixation test with it yields a more sensitive reaction, and a negative result, therefore, should carry more weight in excluding syphilis; furthermore, the chances for non-specific or falsely positive reactions are much less than in tests employing serum.

Cerebrospinal Fluid Examinations of Value in the Diagnosis and Treatment of Neurosyphilis.—From the standpoint of easily applied tests, the following are of value: (*a*) changes in pressure; (*b*) changes in physical appearance; (*c*) total number and kinds of cells; (*d*) chemical changes with special reference to proteins and sugar; (*e*) serological changes with special reference to the presence of antisheep hemolysin (Weil-Kaffka reaction), the complement-fixation, colloidal gold and colloidal mastic reactions. It is my custom to record the pressure, amount of fluid removed, physical appearance of the fluid, total cells per cubic millimeter, etc., on all cases examined, the chart shown (Chart 5) devised for these records, being found very useful. But in a treatise of this kind space does not permit of a detailed discussion of the technic and diagnostic significance of these tests although brief mention may be made of the two most valuable ones, namely, the complement-fixation and colloidal gold reactions.

Cerebrospinal fluid for these examinations is usually secured by spinal puncture in the lumbar region, the technic being described in Chapter LXI. It is possible that fluid secured at this level is not representative of the whole cerebrospinal fluid as indicated by the investigation of Solomon and Welles,[21] who found marked differences in the cytology, chemistry and serology of ventricular and lumbar sac fluids in the same cases; Schoenfeld[22] found no differences in fluids drawn off at four different levels between the eleventh dorsal and fifth lumbar vertebrae. Experience has shown that examination of the fluid secured by lumbar puncture yields extremely valuable diagnostic data and is the best available for routine examinations.

Wassermann Reaction with Cerebrospinal Fluid in Syphilis, with Special Reference to the Kolmer Complement-fixation Reactions.—One of the most valuable tests for neurosyphilis is the complement-fixation test with cerebrospinal fluid. The Sachs-Georgi, Kahn and other precipitation tests, while technically simpler, have not proven sufficiently sensitive. In the original Wassermann test the spinal fluid was employed in dose of 0.4 c.c., but we now know that this amount was too small and that with this technic doses as high as 1.5 c.c. may be employed with perfect safety and greatly increase the percentage of true positive reactions.

CHART 5.—CHART USED BY THE AUTHOR FOR RECORDING THE RESULTS OF CEREBROSPINAL FLUID EXAMINATIONS

CEREBROSPINAL FLUID EXAMINATION

NAME: AGE: PHYSICIAN: DATE:
CLINICAL DIAGNOSIS:

PRESSURE (MILLIMETERS OF MERCURY)	AMOUNT REMOVED (C.C.)	PHYSICAL PROPERTIES	CELLS PER C. MM.	DIFFERENTIAL CELL COUNT			PROTEIN TESTS						COMPLEMENT-FIXATION REACTION	
							QUALITATIVE	QUANTITATIVE						
				LYMPH.	POLYS.	ENDOTHEL.	PANDY	0.2	0.1	0.05	0.025		BLOOD	SPINAL FLUID
Before.....														
After.....														

COLLOIDAL GOLD AND MASTIC REACTIONS*

REGISTRY No.	COLOR REACTIONS	DILUTIONS OF SPINAL FLUID										REMARKS
		1 1:10	2 1:20	3 1:40	4 1:80	5 1:160	6 1:320	7 1:640	8 1:1280	9 1:2560	10 1:5120	
5	Colorless.....											
4	Pale Bule.....											
3	Blue.....											
2	Lilac or purple.....											
1	Red-blue.....											
0	Red-unchanged.....											

*_____ gold curve; .....mastic curve.

Smears:
Dextrose:
Quantitative Kolmer Reaction:
Serum 0.1 c.c.
Serum 0.05 c.c.
Serum 0.025 c.c.
Serum 0.005 c.c.
Serum 0.0025 c.c.

Bacteriological Examination:
Culture:

Animal Inoculation:
Quantitative Chlorids:
Quantitative Kolmer Reaction:
Spinal Fluid 0.5 c.c.
Spinal Fluid 0.25 c.c.
Spinal Fluid 0.125 c.c.
Spinal Fluid 0.0625 c.c.
Spinal Fluid 0.03125 c.c.

EXAMINED BY:

In my new method a dose of 0.5 c.c. of spinal fluid corresponds to about 1.5 to 2.0 c.c. in the original Wassermann test, just as 0.1 c.c. of serum in my method corresponds to 0.3 to 0.4 c.c. in the original test, when calculations are based upon the relative amounts of complement employed in each. In my new method the first dose is 0.5 c.c., which greatly increases the sensitiveness of the reaction; indeed, one may use 1 c.c. of fresh fluids with safety and increase the specific sensitiveness of the reactions to a slightly greater degree. Apparently non-specific reactions are not nearly as likely to occur with spinal fluid as with serum, and, since they may be safely employed in these larger amounts, spinal fluid tests frequently yield positive reactions when the serum tests yield negative reactions.

As stated in Chapter XXXIX, tests employing single doses of serum may occasionally yield falsely negative reactions, because of the presence of some substance in serum tending to inhibit complement-fixation by "reagin" and antigen; with such sera, positive reactions occur with smaller amounts of serum. This phenomenon, however, has not been observed with spinal fluids and it is far safer, therefore, to use single large doses of spinal fluid in conducting complement-fixation tests than single large doses of serum. A quantitative test, however, employing varying amounts of spinal fluid, is to be preferred, as yielding reactions of greater value for estimating the degree of complement fixation. For this reason I employ five different amounts of fluid ranging from 0.5 c.c. to 0.03 c.c. shown in Chart 5. The reactions are expressed as *negative*, if hemolysis is complete in all tubes; *weakly positive*, if fixation occurs in the first tube; *moderately positive*, if fixation occurs in the first two tubes; *strongly positive*, if fixation occurs in the first three tubes and *very strongly positive*, when fixation occurs in the first four or all five tubes. The degree of fixation in each tube may be expressed with the ++++, +++, ++, and + method as in the usual qualitative test; the method gives, therefore, a two-way quantitative reading as described with more detail in Chapter XXXIX.

Undoubtedly the complement-fixing antibody, or "reagin," is produced locally in the syphilitic foci in the central nervous system. Wassermann and Lange[23] have expressed the opinion that one source is the small lymphocytes. They have found that autolysis of these cells in spinal fluids from syphilitic cases increases complement fixation, whereas autolysis of the cellular elements in fluids from non-syphilitic cases (non-syphilitic meningitis and alcoholic dementia) did not produce these substances. It is highly probable, therefore, that in some cases of neurosyphilis the "reagin" content of the spinal fluid is higher than of the serum, but, since spinal fluid is being rapidly absorbed, I believe the occurrence of positive reactions with cerebrospinal fluid with negative serum reactions are largely to be explained on the fact that larger amounts of the former may be employed in the tests.

A question of great practical significance, however, is whether "reagin" in the blood may gain access to the spinal fluid. In other words, does a positive spinal fluid reaction indicate spirochetic infection of the central nervous system, or may it be due to the passive transfer of the "reagin" in the blood plasma produced elsewhere in the body? According to the experiments of Sekiguchi and myself,[24] it is apparently possible for syphilis "reagin" in the blood to appear in traces in the spinal fluid, because the spinal fluids of dogs tested three hours after the intravenous injection of 30 to 50 c.c. of human syphilitic serum per kilogram of weight sometimes yielded weakly positive reactions. After the intraspinal injection of an irritant as sterile horse serum, the amount of "reagin" in the spinal fluid was somewhat greater. A positive complement-fixation reaction with spinal fluid, showing no increase of cells and no increase of protein, or but a trace, may be due to a passive transfer of reagin in cases presenting strongly positive serum reactions; but in the great majority of cases the positive complement-fixation reaction is accompanied by at least a slight increase of cells and protein and is to be interpreted as indicating spirochetic invasion and infection of the tissues of the central nervous system.

When the spinal fluid is tested during the primary and early secondary stages of syphilis, the complement-fixation reactions are frequently negative when the cells and protein are slightly increased. This indicates that spirochetic invasion first brings about an increase of cells and protein, but that more time is required for the production of sufficient "reagin" to yield positive reactions.

As with serum tests, the specificity and sensitiveness of the technic are of primary importance in examinations of spinal fluid. With my new method, the reactions are frequently positive before the colloidal gold and mastic tests yield positive reactions, and I believe the complement-fixation test is the most reliable single test for syphilis of the central nervous system and especially with spinal fluids containing traces of blood, which render cell counts, protein determinations and the colloidal gold reactions unreliable or worthless. In other words, a trace of blood in spinal fluid does not interfere with the complement-fixation reaction, and under acceptable technical conditions it is diagnostic of syphilis, at least in this country, where frambesia (yaws) and trypanosomiasis do not occur, these being the only two non-syphilitic diseases capable of producing positive reactions.

Variations in Serum- and Spinal-fluid Complement-fixation Reactions.—In neurosyphilis the blood serum and spinal fluid Wassermann reactions may present varying results which are of considerable importance in relation to diagnosis and treatment and frequently troublesome from the standpoint of proper interpretation. Four combinations may occur as follows:

Both positive.
Both negative.
Blood serum, positive; spinal fluid, negative.
Blood serum, negative; spinal fluid, positive.

As would be expected, the percentages of the four groups observed by different investigators have varied considerably according to the stage of syphilis, the treatment given and especially the sensitiveness of the complement-fixation method.

As previously stated, I believe that the differences are sometimes due to technical factors and that we are better able to detect traces of complement-fixing "reagin" in the spinal fluid than in the serum, because we may safely use in our tests at least five times more of the former than of the latter, thereby accounting for some of the cases in which the blood serum reaction is negative and the spinal fluid positive.

Both Blood-serum and Spinal-fluid Reactions Positive.—In untreated early syphilis the blood reactions are positive in 85 to 100 per cent. of cases, but the spinal-fluid reactions are positive in but 30 to 40 per cent. In treated cases of early syphilis, the blood-serum reactions are positive in from 80 to 90 per cent. and the spinal-fluid reactions in 20 to 30 per cent. Both are positive, therefore, in but 20 to 40 per cent. of cases of early syphilis. This variation in blood- and spinal-fluid reactions in early syphilis is due to the fact that in the tissues of the central nervous system spirochetic infection has not occurred or is too early or too light to produce in the spinal fluid appreciable amounts of "reagin."

In late or chronic neurosyphilis syphilis, however, there is greater uniformity in the blood-serum and spinal-fluid reactions, because of the infection of the central nervous system; not infrequently, however, the blood serum reaction will be negative and the spinal-fluid positive, on account of the fact that "reagin" is being produced in larger amount in the nervous system and finding its way into the spinal fluid.

Both Blood-serum and Spinal-fluid Reactions Negative.—This combination is particularly influenced by the sensitiveness of the complement-fixation method. In untreated syphilis in the late primary stage, the blood serum may give a negative reaction in about 15 per cent. of cases with my method, and in these the spinal-fluid reactions are likewise invariably negative. In the secondary stage, however, the serum reactions are positive in almost 100 per cent. and the spinal-fluid reactions positive in about 40 per cent. In treated cases of early syphilis, about 10 to 20 per cent. may give negative reactions with both blood serum and spinal fluid. In chronic neurosyphilis however, it is very difficult to express percentages and I have not yet accumulated sufficient data with my new method upon which to express an opinion.

Blood Serum Positive and Spinal Fluid Negative.—This combination is particularly likely to occur in early cases of syphilis and especially in treated cases. For example, in cases examined within one year after infection and receiving no treatment, the blood-serum reactions are 85 to 100 per cent. positive and the spinal-fluid reactions 30 to 40 per cent. positive; in treated cases, the serum reactions are 80 to 90 per cent. positive and the spinal-fluid reactions 20 to 30 per cent. positive.

In chronic neurosyphilis, however, the blood serum is seldom positive with negative spinal-fluid reactions, provided large amounts of spinal fluid are employed. If relatively small amounts of spinal fluid are tested, the proportion of positive serum reactions will be considerably higher and especially in tabes dorsalis. But if the proportions of serum and spinal fluid employed in the tests are 1:5 or 1:10, not more than 10 per cent. of cases of chronic neurosyphilis will yield a positive serum and negative spinal-fluid reaction.

Blood Serum Negative; Spinal Fluid Positive.—This group is of special interest and importance in neurosyphilis and particularly in cases of syphilis receiving treatment. As a general rule, the spinal fluid is not examined until the blood serum reactions have been rendered negative by treatment; but in about 15 to 25 per cent. of cases of syphilis in which treatment is given and negative blood-serum reactions are observed within the first eighteen months after infection, the spinal-fluid reactions will be found positive and an indication for further specific treatment. Of course, the percentage of such cases varies with the kind of treatment given in the early stages of syphilis, but the possibility of their occurrence constitutes a demand for at least one examination of spinal fluid after the first year or eighteen months of treatment, to guard against the very regrettable error of discharging a patient as cured on the basis of even a series of negative blood-serum reactions. And there is no reliable clinical substitute for spinal fluid examinations, as such cases may present absolutely no neurological signs or symptoms; not even pupillary changes.

Lange Colloidal Gold Reaction with Cerebrospinal Fluid in Syphilis.—The colloidal gold or gold sol reaction with cerebrospinal fluid is one of the most valuable tests available for neurosyphilis. Unfortunately, however, its reactions are not specific for syphilis and may occur in some non-syphilitic diseases associated with meningitis or parenchymatous changes with nerve tissue destruction. But in the majority of cases of syphilis, the possible presence of these disturbing factors are readily eliminated and under these conditions the colloidal gold test serves as a sensitive detector of neurosyphilis and the kind or degree of reaction furnishes an index of the degree of infection. One distinct disadvantage of the Lange colloidal gold reaction, however, is the difficulty of preparing the reagent. If this is too sensitive falsely positive reactions may occur; if the colloidal gold is too greatly protected, the reagent is not sufficiently sensitive and yields falsely negative reactions. I have repeatedly sent specimens of the same spinal fluid to three different laboratories and obtained widely varying reports. We are urgently in need of a standardized method for preparing the reagent, so that different lots prepared at different times may yield closely similar results. Until such is available, I believe it is hardly worth while employing the colloidal gold test as a guide in treatment, because with different reagents in apparently perfect physical condition one may obtain widely varying results with the same spinal fluid. The presence of blood in spinal fluid also opens up an avenue for error in this test which is absent in the complement-fixation test.

With an acceptable reagent, however, the colloidal gold test is very valuable and should not be omitted in the routine examination of spinal fluid for syphilis. As shown in Chart 5, ten dilutions are employed prepared with 0.4 per cent. saline solution and the technic of setting up the tests is extremely simple. The reagent has a deep red color and, when this is unchanged, the reaction is expressed with a cipher, so that a line of ten ciphers (0000000000) indicates a negative reaction. The first evidence of precipitation is indicated by a change in color to red-blue indicated by 1 and from this to complete precipitation, indicated by 5, there is a range of colors as listed in Chart 5. In paresis the precipitation is usually complete in the first three to six tubes, giving a color reaction something like 5555321000; this type of curve is designated as the "paretic curve" or Zone I reaction. In tabes the color changes are usually somewhat as follows: 0135521000 designated as the "tabetic curve," "leutic zone reaction" or Zone II reaction.

Unfortunately, however, these types of reactions are not pathognomonic. The "paretic curve" is generally found in paresis or in the pre-paretic stage of neurosyphilis, but may occur in syphilitic meningitis (cerebrospinal syphilis) and even in early asymptomatic neurosyphilis as reported by Moore.[25] As shown by Fordyce, however, a "leutic zone" or Zone II reaction constitutes very reliable evidence against the presence of paresis and in this manner the colloidal-gold reaction is frequently of great value in differential diagnosis. Fordyce, Wile and others, have sometimes observed Zone I or "paretic curves" many months before the development of even the early clinical symptoms of paresis, and the reaction may give, therefore, warning of the threatened onset of this dreaded disease and indicate thereby the necessity of vigorous treatment. This type of curve is always diagnostic of syphilis and is always to be interpreted as of very grave prognostic significance. Individuals whose spinal fluid yields a reaction of this kind are to be carefully watched for paretic symptoms and signs, and should receive intensive treatment; this curve is usually associated with an increase of cells and protein and strongly positive complement reactions, and, as stated above, these are invariably grave cases of neurosyphilis, apt to be "Wassermann-fast" and demanding the utmost vigilance and diligence in treatment.

The second type of curve, the so-called "luetic," "tabetic" or Zone II reaction, indicates the presence of neurosyphilis, but bears no constant relation to the signs and symptoms. It is usually found in asymptomatic cases and indicates the presence of syphilis in just the same manner as a positive Wassermann reaction; while the "paretic" or Zone I reaction is highly diagnostic of syphilis, this so-called "luetic" or Zone II reaction may occur in non-syphilitic diseases of the central nervous system and, when the spinal fluid yields a negative complement-fixation reaction and is otherwise approximately normal, the reaction has little or no significance.

In syphilis this type of curve has a much better prognostic import than the "paretic" or Zone I curve. Such patients are not as heavily infected, are not apt to be "Wassermann-fast" and respond more promptly to treatment. The outlook for the patient is always more favorable than when the spinal fluid yields a "paretic" or Zone I reaction.

Time of Invasion of the Central Nervous System with Spirochæta Pallida. There is little or no doubt that in practically all untreated cases of syphilis in the primary and secondary stages, the tissues of the central nervous svstem are invaded with *Spirochæta pallida* during the initial spirochetemia; evidences are found in the spinal fluids of approximately 60 per cent., but in the balance it is highly probable that invasion has occurred without the production of symptoms or spinal-fluid changes.

The question now arises, may invasion occur at subsequent times? If the spinal fluid is found normal one or two years after infection of cases receiving none or insufficient treatment, does this guarantee that the central nervous system is free of infection and will remain free? Unquestionably infection of the central nervous system is successfully combated by some patients who are insufficiently treated or not treated at all; but does this initial escape protect against subsequent infection? Most writers have expressed the opinion that if the spinal fluid shows no abnormalities at the end of the first or second years after infection, that neurosyphilis is not likely to develop at a subsequent time; indeed, some have stated that if the spinal fluid is found normal after the first or second courses of treatment, that neural infection will not subsequently occur and that further spinal-fluid examinations are not required. Wile and Marshall[26] believe that any patient whose spinal fluid is found normal after the secondary eruption has developed is

not likely to subsequently show abnormal changes; Stokes and McFarland[27] observed only four cases in a series of 114 who developed abnormal spinal-fluid changes after preceding negative examinations, and Finger and Kryle[28] have likewise stated that while there is no relation between the degree of cutaneous exanthems and percentage of meningeal invasions, yet negative spinal-fluid examinations at the end of the secondary period of syphilis tells the story and that a reversal to abnormal changes in the later stages is rare. As stated by Stokes and McFarland, "the syphilographer who carries the treatment of his primary and secondary cases into their second course without examination of the spinal fluid is proceeding blindfolded."

I believe it is true that, if the spinal fluid is found normal at the end of the first year of treatment, the patient can be greatly assured that the danger of neurosyphilis is past, but this certainly is not true after but one or two courses of treatment. Even after one and two years of treatment, complete cure may not be effected and there is always danger of temporary periods of spirochetemia with invasion and possible infection of the central nervous system. Experience has taught me in common with others that the greatest danger of neural infection is during the early stages of syphilis, that is, during the first six to twelve months after infection, but as long as the blood Wassermann reaction is positive or other signs of uncured syphilis are present, I believe that the danger of development of neurosyphilis exists. For this reason a negative spinal fluid examination with a positive blood Wassermann reaction does not assure against the possibility of subsequent neural infection; only when a negative spinal-fluid examination follows a series of negative Wassermann reactions over a period of one or preferably two years, may we feel reasonably assured that neurosyphilis will not subsequently develop. For this reason I routinely advise spinal puncture after the first six months or year of treatment to be followed by at least one more examination after the blood complement-fixation reaction has been persistently negative for one or two years.

Influence of Chemotherapy upon Cerebrospinal-fluid Changes in Early Syphilis.—The very important influence of specific treatment in the early stages of syphilis on the cerebrospinal-fluid abnormalities and clinical manifestations of neural infection has been brought out in a clear and convincing manner by the investigations of Fordyce and Rosen, Wile, Stokes and McFarland, Keidel, Moore and others mentioned above. Of course, the findings of these investigators have varied considerably and especially because the amount and kind of treatment given the respective group of patients have varied, but there is a uniformity of opinion in support of the statement that after three or more courses of treatment, each course embracing not less than four to six injections of arsphenamin or neoarsphenamin at weekly intervals followed by four to six weeks of mercury by injection or inunction, the incidence of abnormal spinal fluid findings is reduced almost one half. For example, in primary syphilis, the incidence is reduced from 20 to approximately 12 per cent. by arsphenamin treatment and in secondary syphilis, from 40 to 60 per cent. to approximately 25 to 35 per cent. According to Stokes and McFarland, the percentage of abnormal spinal fluids among cases of early syphilis may be reduced from 60 to 70 per cent. to 40 per cent. within the first six months, and to 25 or 30 per cent. after the first year or two, the decline being more gradual from this time.

As shown by Keidel and Moore,[29] treatment must be continuous over a comparatively long period of time to exert these effects. A few injections of arsphenamin or neoarsphenamin with or without mercury, accomplishes but little. As shown by Moore, the incidence of 29.8 per cent. of neurosyphilis

in one group with little treatment was reduced to 13.1 per cent., in a second group treated with eighteen doses of arsphenamin plus mercury, and to 8.0 per cent. in a third group treated with twenty-four or more doses of arsphenamin plus mercury. A decrease in cells is usually the first sign of improvement followed by a decrease of protein, a reduction in the degree of complement-fixation and finally a reduction in the degree of colloidal gold precipitation. The last mentioned reaction is the last to disappear, and in my experience, fluids showing the "paretic" or Zone I type of reaction are seldom influenced to any marked degree except in some cases treated by malarial inoculation.

But the response to treatment varies considerably among different cases of early neurosyphilitics. For example, among those presenting no symptoms but showing an increase of cells and protein with negative complement-fixation and colloidal gold reactions, the response is far more marked than among those showing some symptoms, pleocytosis, increase of protein and positive complement-fixation and colloidal gold reactions; indeed, among this group four courses of treatment may have little or no effect upon the Wassermann and gold reactions, although symptoms disappear and the pleocytosis and protein content decrease to normal or approximately normal proportions. Furthermore, as a result of treatment, the spinal-fluid changes may be temporarily increased, probably as a result of producing neuro-recurrences which I shall discuss shortly with more detail, but producing no signs or symptoms of such, because, as suggested by Gennerich,[30] the lesion is in a "silent area" not involving cranial nerves or other important tissues.

While these results show the very considerable importance of active and continuous specific medication in the early stages of syphilis for the prophylaxis and cure of neural invasion and infection, actual harm may be done by insufficient treatment. Moore has shown, for example, that, when treatment in early syphilis voluntarily ceased or "lapsed" after one to twelve doses of arsphenamin, either with or without mercury, and the spinal fluid was examined weeks or months later, the incidence of abnormal spinal fluids was three times higher than under regular treatment. Furthermore, as a result of lapsed treatment, the percentage of cases showing the advanced spinal-fluid changes of high pleocytosis, increase of protein and positive complement-fixation and colloidal gold reactions with slight symptoms, became twelve times higher than a similar group under regular treatment.

Irregular treatment, therefore, may be even worse than no treatment at all, insofar as invasion and infection of the central nervous system is concerned, because the incidence of infection may be actually higher than when no treatment at all has been given. The reasons for this are probably the same as advanced for explaining the mechanism of neuro-recurrences under arsphenamin treatment, which are briefly discussed in the succeeding section.

Influence of Chemotherapy upon the Production of Neuro-recurrences.—Soon after the introduction of salvarsan for the treatment of syphilis, it was noticed that not infrequently precocious clinical neurosyphilis followed the administration of one or several doses. The subject naturally excited a great deal of discussion as regards the nature and mechanism of the reaction and for a while it was regarded as a toxic effect of salvarsan upon nerve tissue or an expression of neurotropism of this substance. But it is now definitely known to be due to *Spirochæta pallida* and the tissue changes excited by this parasite, an excellent bibliography of the literature on the subject prior to 1912 being given by Ellis[31] along with the report of a case developing during the secondary stage of syphilis.

Almost without exception neuro-recurrences (neuro-recidives) develop as a result of lapses in treatment after a few doses of arsphenamin or neoarsphenamin. Ehrlich[32] believed that, under these conditions, when arsphenamin treatment fell short of complete sterilization, spirochetes survived in isolated foci and became even more active than usual because the treatment given not only failed to sterilize but reduced the development of resistance or immunity by the patient. In these foci the surviving spirochetes produce an intense lymphoid infiltration and, if they happen to be situated in physiologically important tissues as one or more of the cranial nerves, marked signs and symptoms develop; on the other hand, if the foci are situated in the "silent areas" or physiologically unimportant tissues, signs and symptoms of these neuro-recurrences may not be apparent.

As a general rule, the lesions of neuro-recurrences develop rather slowly and occur weeks or months after the interruption of specific medication with arsphenamin, neoarsphenamin or mercury. In this respect they differ from Jarisch-Herxheimer reactions. The latter usually develop in a matter of hours or a few days after an injection of arsphenamin or neoarsphenamin and are expressed by an exacerbation of lesions or symptoms already present; neuro-recurrences, however, are new lesions developing slowly rather than the sudden accentuation of present lesions. Neuro-recurrences are due to the renewed activity of spirochetes in a host who has not been able to develop sufficient immunity; Herxheimer reactions suggest rather a reaction between medicament and syphilitic tissue, although they may be due to renewed spirochetic activity or result in the stimulation of spirochetes.

Spinal fluid changes generally occur upon the development of a neuro-recurrence and especially an increase of the cells, but this pleocytosis does not necessarily indicate a diffuse meningeal reaction, but may result from one or several focal lesions. Diagnosis is largely based upon clinical signs and symptoms and usually offers no difficulties.

From the standpoint of chemotherapy, emphasis is to be laid upon the value of continuous treatment of early syphilis for the prophylaxis of neuro-recurrences; treatment of the nerve lesions themselves is considered in Chapter LXXVI. Some physicians hesitate to administer arsphenamin and neoarsphenamin in the *early stages* of syphilis believing that the development of immunity is thereby retarded; mercury is first administered followed by arsphenamin or neoarsphenamin. However, if treatment is vigorous and continuous after the plan described in Chapter LXXI, the danger of neuro-recurrences or precocious neurosyphilis is reduced to a negligible minimum as the spirochetes are rapidly destroyed; I believe it is a dangerous principle to rely too much upon the development of immunity for the aid it may render in protecting tissues and organs. Rather it is advisable to destroy the spirochetes as promptly as possible if the general condition of the individual case permits of the vigorous administration of arsphenamin or neoarsphenamin followed by mercury.

Others prefer starting the treatment of *chronic* syphilis with mercury in order to avoid sudden stimulation of spirochetes with the more powerful arsenical preparations; I believe this is a good practice, as discussed in Chapter LXXIV.

Spinal Fluid Changes in Chronic Syphilis and the Influence of Chemotherapy upon These.—In chronic latent and active syphilis, changes in the cerebrospinal fluid are usually to be found but not always; for this reason a normal spinal fluid may be found in the presence of neural infection, but the lesion or lesions are likely to be vascular, small, inactive and asymptomatic. In this connection may be mentioned the studies of Solomon and Klauder[33]

upon the negative spinal fluid findings in cases of neurosyphilis and especially those with vascular lesions; likewise the report of Southard and Solomon[34] of negative spinal-fluid findings in cases presenting clinical symptoms and signs of paresis, "paresis sine paresi," and the report of Wynn[35] concerning the disappearance of spinal-fluid findings in a case of paresis although with a continuation of symptoms. Mention may be made also, at this time, of the report of Solomon and Klauder,[36] indicating that the intravenous or intraspinal injection of arsphenamin may provoke positive spinal-fluid changes in cases previously negative.

Not infrequently only a slight increase of protein and a slight increase of cells are the only abnormalities found in the spinal fluid, and these are frequently over looked or under rated and ascribed to technical inaccuracies. Their occurrence emphasizes anew the necessity for accuracy in the conduct of spinal fluid examinations, so that due notice and emphasis may be placed upon even the slight abnormal changes.

Can spirochetes be present in the tissues of the central nervous system without producing symptoms, changes in the spinal fluid or positive blood Wassermann reactions? This very important question of course bears a relation to the question of complete cure of syphilis and cannot be answered at the present time on the basis of proven facts. At least one or more decades must pass during which we may watch and study patients with all these present-day criteria of "cure" to determine if cure has been absolute. I am prepared to believe, however, that it is possible for spirochetes to be so reduced in numbers and activity as to remain quiescent in the neural tissues without the production of spinal fluid and other changes, as Warthin has apparently shown may occur in the myocardium and other internal organs. But I am quite sure that under these circumstances "cure" may be said to exist, insofar as general health and usefulness are concerned, and that active lesions usually produce sufficient changes in the spinal fluid to indicate their presence even though symptoms are not produced, or are light and atypical.

As would be expected, the spinal-fluid changes in chronic syphilis vary considerably in different patients according to the size, location and activity of the lesions. Even among individuals presenting the same symptom-complexes and suffering presumably with the same kind of lesions, the spinal fluid changes may vary, but I cannot devote space to a consideration of these, since my purpose is to deal mainly with the treatment rather than the diagnosis of syphilis. From the standpoint of "typical" changes in the spinal fluid, probably Chart 6 may prove of some aid in diagnosis, in which the changes have been arranged according to the anatomical and clinical classification of neurosyphilis given on page 536.

I have not recorded in this chart the findings in syphilitic psychoses, because relatively few have come under my observation; Fordyce states that the spinal fluid is usually normal. A word may be said, however, regarding these cases. Due care must be exercised against including cases of "syphilophobia" in this group; also that relatively large number of syphilitics with what Collins[37] has so aptly expressed as having "syphilitic scars of the spirit." Many cases of syphilis develop this pessimism regarding cure and ascribe every ache and pain to syphilis regardless of how ridiculous their fears are in many instances. Such a condition may develop into a psychosis, but I reserve the term for those mental states sometimes developing in tabes and other forms of neurosyphilis which may have an actual organic basis.

Under the influence of specific medication, the cerebrospinal-fluid changes are modified in some cases, while in others no change occurs or they increase in severity. Owing to the pain and discomfort of spinal puncture and par-

CHART 6.—CHART SHOWING TYPICAL LABORATORY FINDINGS IN NEUROSYPHILIS

DIAGNOSIS	CEREBROSPINAL-FLUID CHANGES					POSITIVE BLOOD WASSERMANN REACTIONS, PER CENT.
	PRESSURE	TOTAL CELLS	PROTEIN	POSITIVE WASSERMANN REACTIONS, PER CENT.	POSITIVE COLLOIDAL GOLD REACTIONS,* PER CENT.	
Early† asymptomatic, untreated	Not increased	2–150	+ to +++	30– 40	15 Zones I and II	85–100§
Early asymptomatic, treated	Not increased	0– 100	+ to ++	20– 30	12 Zones I and II	80– 90
Early acute meningitis	Increased	100–1000	++++	100	100 Usually Zone I	100
Chronic‡ latent asymptomatic	Not increased	0– 20	− to +	60– 80	60–80 Usually Zone II	50– 80
Chronic diffuse meningitis (cerebrospinal syphilis)	Increased	5– 500	+ to ++++	90–100	90–100 Usually Zone I	60– 80
Chronic localized meningitis	Not increased	0– 40	+ to ++	80– 95	80– 90 Usually Zone II	80– 95
Paresis	Increased	10– 300	++ to ++++	100	100 Usually Zone I	95–100
Tabes dorsalis	Usually not increased	20– 200	+ to ++	90– 95	90–100 Usually Zone II	80– 90
Tabo-paresis	Increased	10– 200	+ to +++	90–100	90–100 Usually Zone I	90–100
Erb's spastic spinal palsy	Not increased	5– 50	+ to ++	80– 90	80– 90 Usually Zone II	80– 90

* Zone I refers to the "paretic" type of curve (example: 5553210000); Zone II refers to the "luetic" zone curve (example, 0134420000).
† "Early" refers to cases examined within 12 to 18 months after infection.
‡ "Chronic" refers to cases examined 1½ years or longer after infection.
§ Eighty-five per cent. positive Kolmer complement-fixation reactions in late primary stage; 100 per cent. positive in secondary stage.

ticularly the chances of developing spinal puncture headache, we are not usually able to conduct more than one to three spinal punctures unless a form of intraspinal treatment is being employed.

Apparently the first sign of improvement is a decrease in cells, although, if spinal injections are being given, the cells may be temporarily increased due to meningeal irritation. The next change is a decrease in proteins accompanied by a reduction in the degree of complement fixation. The last change to be affected is the colloidal gold reaction and it is usually the last abnormality to disappear. Zone II reactions are more readily influenced than Zone I and may be eradicated altogether. Zone I reactions ("paretic curves") may be converted in to Zone II reactions ("leutic zone curves"), but in my experience are rarely eradicated altogether by medicinal treatment although sometimes disappearing under treatment by malarial inoculation.

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PART VIII

PHARMACOLOGY AND TOXICOLOGY OF ANTISYPHILITIC MEDICAMENTS

CHAPTER XLIII

PHARMACOLOGY OF ARSPHENAMIN, NEOARSPHENAMIN AND ALLIED DRUGS IN RELATION TO METHODS OF ADMINISTRATION AND THE TREATMENT OF SYPHILIS

Since syphilis of human beings is predominently an infection of the fixed tissues rather than of the body fluids, it is required as a principle of treatment that the chosen remedy shall be present in adequate amounts and in an *active* spirocheticidal state over relatively long periods of time with a minimum degree of toxic effects. In the very earliest stages of syphilis medicaments may readily reach and destroy the spirochetes in the tissues and lymph spaces of the chancre and likewise those organisms already in the lymph, lymphatic glands and blood; but after dissemination has become widespread, with lymphocytic and fibrous tissue infiltrations about the foci of spirochetes, the matter of penetrability of these inflammatory exudates by the organic arsenical, mercurial or bismuth compound chosen for treatment, requires careful consideration. In this relation the size of the dose, frequency and route of administration are of primary importance in which clinical experience and a knowledge of the results of animal experiments for curative and toxicological effects, absorption, distribution and elimination of the drug, is required in the interests of best therapeutic management and especially in the chronic stages of the disease. The rapidity of absorption of the drug is important in relation to the question of prolonged therapeutic activity; for this reason it has long been recognized that a therapeutically active organic arsenical compound which may be administered by subcutaneous or intramuscular injection would have a very useful place in the treatment of chronic syphilis, and sulpharsphenamin (sulfarsenol) has been recently advocated for this purpose. Rapidity of elimination is likewise a factor of importance, but with the organic arsenicals this refers more to the elimination of the therapeutically active oxidation products of the arsenobenzol compounds than to the elimination of the inorganic arsenic in the form of arsenites and arsenates. In other words, the rapidity of elimination of the therapeutically active "oxids" of the organic arsenical compounds and the pentavalent arsenicals bear an important relation to the degree and duration of therapeutic activity, while elimination of the arsenic of these compounds in inorganic form is of lesser interest because these arsenites and arsenates possess little or no therapeutic activity. However, the elimination of these bears an important relation to the question of toxicological effects.

For these and other reasons studies bearing upon the absorption, distribution, retention and elimination of arsenical, mercurial, bismuth and

iodin compounds have an important practical relation to the question of maintaining the medication in the body fluids at the point of maximum therapeutic activity with the minimum toxicological effects for sufficient periods of time to bring about the destruction of *Spirochæta pallida* in the blood, lymph and fixed tissues; in this chapter it is proposed to discuss these phases of the pharmacology of the organic arsenical compounds in relation to the methods of administration and the treatment of syphilis.

Importance of Analytical Methods and of "Normal" Arsenic in Tissues and Excretions.—Owing to the great interest for centuries in arsenic from the toxicological standpoint, numerous methods have been devised for its quantitative estimation in the tissues and excretions. Soon after the discovery of salvarsan and its use in the treatment of syphilis, untoward effects stimulated renewed interest in the question of the fate of the drug after intramuscular and intravenous injection with particular reference to the elimination and retention of arsenic. Abelin[1] devised a method for the detection of arsphenamin and its substitutes in the blood, urine, etc., but most investigators have employed methods for the qualitative and quantitative estimations of inorganic arsenic. To-day considerable difference of opinion exists among chemists regarding the sensitiveness and comparative value of these methods; probably the Reinsch, Marsh and Gutzeit methods or modifications of these have been mostly employed, although within recent years the Gutzeit, Marsh-Berzelins and the Green iodometric methods appear to have been proven especially reliable and popular.

From the standpoint of arsenic determinations in the excreta and tissues, the question of the presence of this element "normally" has commanded considerable attention, since Gautier[2] in 1899 asserted that arsenic is a normal and consistent constituent of the thyroid, thymus and mammary glands, blood, brain, skin, bones, hair and milk of man and various animals. The quantities found were very small, being less than 0.75 mg. for each hundred grams of tissue and the content of the blood not exceeding one part in fifty millions. These observations were soon confirmed by Bertrand,[3] Schaefer,[4] Knecht and Deardon[5] and others, and in 1903 Gautier and Clausmann[6] found traces of arsenic in a variety of foods such as meats, milk, eggs, fish, vegetables, wines, etc., from which they estimated that each inhabitant of Paris receives about 7.66 mg. arsenic per year in his food. Bloemendal,[7] Bang[8] and Klason[9] have found arsenic in normal urine, and the sum total of these investigations have shown the great importance of taking into consideration the possibilities of this so-called "normal or physiological" arsenic not only in medico-legal determinations, but also in studies concerning the fate of arsenic after the administration of arsenical compounds.

Within the last year Fordyce, Rosen and Myers,[10] employing a modification of the Marsh test,[11] have studied this question of "normal" arsenic with particular care in a series of valuable investigations concerning the fate of arsphenamin and allied drugs employed in the treatment of syphilis. An examination of the blood, urine, hair and milk from about 200 persons has led to the conservatively made statement that arsenic is found "normally" in a large number of persons, depending upon the character of their food, drink, medication and environment. Under conditions of diet restricted in the amount of sweets, vegetables, meat, etc., it may be entirely absent. Some persons did not give a positive test at all times. Arsenic was almost invariably present, in different amounts, in persons applying remedies containing ingredients contaminated with it to inflamed or ulcerating skin surfaces, and to a greater extent in persons receiving the element either by mouth or intravenously, or connected with arsenicals in a preparative way. At any rate, this latest analytic data, including such simple, every-day items as rhubarb and soda, sulphur ointment and sulpho-ichthyolate preparations, as well as foods, in the list of "carriers" of arsenic, show that a careful history of the patient is requisite before arriving at a final decision regarding the meaning of the arsenic content of the blood, urine or internal organs.

Absorption of Arsphenamin, Neoarsphenamin and Sulpharsphenamin in Relation to Methods of Administration.—When antisyphilitic drugs are administered by intravenous injection, the question of absorption is of little importance, because the medicaments are thrown into the blood in solution and in the case of the arsphenobenzene compounds may exert parasiticidal effects in a matter of thirty minutes or less. One disadvantage of intravenous

medication, however, is rapidity of elimination and consequently short periods of spirocheticidal activity; likewise toxic effects are more likely to occur.

The rate of absorption after subcutaneous and intramuscular injection, or after administration by mouth or by rectum, is readily determined by chemical analysis of the blood and excreta. In the case of arsphenamin, neoarsphenamin, sulpharsphenamin and allied drugs, examinations of this kind are usually confined to analyses for inorganic arsenic, although direct tests for arsphenamin itself have been applied by means of the Abelin method or some modification of it. The rate and degree of absorption can also be studied biologically, that is to say, by infecting lower animals with trypanosomes or spirochetes at varying intervals after the administration of the drugs. This method has the added advantage of indicating not only the time required for the absorption of the drug, but, furthermore, whether or not the drug has been absorbed in an active parasiticidal condition. The "therapeutic test" is likewise a sort of index of absorption of therapeutically active compounds in the treatment of syphilis of human subjects presenting external lesions that may be carefully watched for regressive changes.

The absorption of antisyphilitic drugs is a question of considerable practical inportance not only in relation to the rapidity but also to the prolongation of curative activity. As we shall shortly review, the rapidity of absorption is influenced by many factors requiring due consideration among which the degree of local inflammatory reaction about the sites of subcutaneous and intramuscular injection, the solubility of the drug, the menstrum employed for suspensions, etc., are the most important.

Absorption after Intramuscular Injection.—The rate of absorption from the muscles depends primarily upon the degree of injury produced; neoarsphenamin, for example, produces much less irritation than arsphenamin and is absorbed almost five to six times more quickly. Some brands of sulpharsphenamin are absorbed even more rapidly. Swift[12] has found that in rabbits injected intramuscularly with arsphenamin in alkaline solution, in neutral suspension and in oily suspension, only about 30 to 40 per cent. absorption of arsenic occurs during the first week, about 50 per cent. during two weeks and that as much as 20 per cent. may still be found in the muscles at the end of six weeks. The rate of absorption was most constant after the injection of neutral suspensions, a little more irregular after the injection of the alkaline solution, and most irregular after the injection of the acid suspension, the results appearing to bear a ratio to the degree of local irritation. The effect of concentration was much less constant. About 80 per cent. of the arsenic of *neoarsphenamin* was absorbed during the first week and 90 per cent. during the first two weeks, about 5 per cent. remaining at the end of six weeks.

Very little data is available at present upon the rate of absorption of *sulpharsphenamin* from the muscles and subcutaneous tissues. My own experiments have been conducted with sulpharsphenamin injected into the thigh muscles of rabbits in doses of 0.1 gm. dissolved in 1 c.c. of sterile distilled water. Analyses and histological sections of the excised muscles were made twenty-four, forty-eight and seventy-two hours later; the Abelin reaction and a modification described by Kolls and Youmans[13] for arsphenamin in the tissues were conducted, as well as total arsenic determinations. The results were quite irregular and varied considerably with two different brands of sulpharsphenamin; the degrees of local irritation varied likewise as discussed in Chapter XLVIII.

As a general rule, the Abelin reactions were positive with muscles excised at the end of twenty-four hours and the determinations for inorganic arsenic showed from 40 to 60 per cent. absorption. At the end of forty-eight hours

the Abelin reactions were generally negative, with from 50 to 80 per cent. absorption; at the end of seventy-two hours the Abelin reactions were negative, with about 90 per cent. absorption of the arsenic. As compared with intramuscular injections of neoarsphenamin in the same dose and concentration per kilogram of weight, the absorption of sulpharsphenamin was generally, but not always, considerably more rapid and likewise produced less tissue injury.

Absorption after Oral and Rectal Administration.—That arsphenamin is absorbed when administered orally was shown by Schamberg and myself[14] in 1912 and since then our observations have been confirmed by others. Arsphenamin and neoarsphenamin have also been administered by rectal injection by some physicians in the treatment of syphilis. Both suppositories and enemeta have been given. Undoubtedly, some absorption occurs after the administration of large doses to human subjects, as shown by curative effects upon syphilitic lesions, but the amount of absorption is somewhat less than follows oral administration and very much less than after intramuscular injection. In my experiments the rectal administration of rabbits with 0.050 gm. arsphenamin per kilogram of body weight in alkaline solution and similar doses of the powder itself in suppositories, did not produce any demonstrable degrees of irritation and traces of arsenic were found in the urine and bile within twenty-four hours, but the amounts were minute and usually none was found after seventy-two hours. Noeggerath and Reichle[15] have studied the absorption of neoarsphenamin after rectal administration to children in doses of 0.6 gm., by applying tests with the urine for the formaldehyd component; only traces could be found, while tests for arsenic in the urine were uniformly negative.

Absorption in Relation to Therapeutic Activity.—As previously stated, the absorption of these organic arsenical compounds may be studied also by biological methods consisting of administering the compounds to rats and infecting them at intervals with trypanosomes for determining the degree of absorption of therapeutically active amounts of drug, according to the curative influence exerted upon the course of the infection, or by administering the drugs to rabbits followed by testicular inoculation with *Spirochæta pallida.* Much more decisive information may be gained, however, in experiments employing rats infected with *Tr. equiperdum,* and the results are directly applicable to the effects produced in *Sp. pallida* infections. In my experiments upon this subject, white rats were infected by intraperitoneal injections of 500,000 *Tr. equiperdum* and twenty-four hours later treated with arsphenamin, neoarsphenamin and sulpharsphenamin by intravenous and intramuscular injection, oral and rectal injection; the results summarized as follows show the single minimal curative doses of various compounds by the four different routes of administration:

Drug	Minimal Curative Doses per Kilogram, by			
	Intravenous Injection	Intramuscular Injection	Oral Administration	Rectal Administration
Arsphenamin	0.004	0.004	0.028	0.045
Sulpharsphenamin	0.020	0.012	More than 0.030	More than 0.060
Neoarsphenamin	0.008	0.010	0.040	0.060

As shown in these experiments, the minimal curative doses of the three different drugs administered by intramuscular injection were practically the

same as by intravenous injection, but 6 to 10 times greater respectively, by oral and rectal administration. I think these results are particularly acceptable as practical indices of the relative therapeutic results to be expected from the organic arsenical compounds administered by these four different routes in the treatment of syphilis of human beings. It is particularly significant that the minimal curative doses of all three compounds by intramuscular injection were practically identical with the minimal curative doses by intravenous injection, in confirmation of the results observed by Voegtlin and Homer W. Smith.[16]

Distribution and Storage of Arsphenamin and Neoarsphenamin in Relation to Methods of Administration.—By whatever method the organic arsenical compounds are administered in the treatment of syphilis, distribution occurs principally in the blood. In intravenous medication, all of the drug administered is, of course, placed at once in the blood; but, as just stated above, arsenic determinations and biological tests have shown that, after intramuscular injections of arsphenamin, neoarsphenamin and sulpharsphenamin, almost as much therapeutically active compound finally reaches the blood by lymphatic and blood absorption as by intravenous injection. In other words, after subcutaneous and intramuscular injections, inflammatory reactions may delay absorption, but it does not appear that more than traces of the drug are fixed or absorbed by the tissues at the site of injection. Furthermore, it does not appear that the local tissues reduce the therapeutic activity of the medicament; indeed, in the case of the organic arsenical compounds, it is highly probable that the production of the active spirocheticidal compounds by oxidation or reduction, as the case may be, according to whether a trivalent or pentavalent compound has been injected, proceeds to some extent in the muscles before absorption and distribution.

After arsphenamin and neoarsphenamin reach the venous blood, they are carried to the heart and after passage through the lungs circulate, for a few hours at least, throughout all vascularized tissues of the body. Doubtless some is retained by the lungs and especially if intravascular precipitates have been produced; indeed, chemical analyses of the lungs of rabbits after intravenous injections usually show that a large amount of arsenic has been retained. Stuhmer,[17] for example, found that, after the intravenous injection of rabbits with acid solutions of salvarsan, most of the drug was to be found in the lungs. He thought that this indicated a special affinity of salvarsan for these tissues, but we now know that the effects were due to the production of precipitates of salvarsan in the blood which were filtered out in the capillaries of the lung. Concentrated alkalinized solutions of salvarsan (1 gm. in 25 c.c.) had a similar effect, whereas neosalvarsan was found to be more widely distributed. Stuhmer observed that neosalvarsan disappeared from the blood within twenty-four hours, whereas the acid and alkaline solutions were retained for as long as three days. He noted such large amounts of salvarsan in the intestinal contents that it was thought possible for oxidation to occur with the production and absorption of toxic substances. The lungs, liver and spleen were found to retain the largest amounts of both compounds, but subsequent investigations have shown that the compounds are not equally retained by the different organs and tissues.

In the first place arsphenamin and neoarsphenamin are distributed largely in the plasma and only a small amount is absorbed or fixed by the corpuscles; Barbat and Myers[18] for example, found more than five times as much arsenic in the blood serum as in the clots within half an hour after the intravenous injection of 0.4 gm. of salvarsan. Clausen and Jeans[19] employing a modification of Gutzeit's method for quantitative arsenic determinations, found

that little or no arsenic was present in the corpuscles after the intravenous injection of arsphenamin and that the serum contained as much as the plasma.

As I shall shortly discuss with more detail in a consideration of the elimination and retention of the organic arsenic compounds, arsphenamin and neoarsphenamin are apparently rapidly eliminated from the blood after intravenous administration. By means of the Abelin test for arsphenamin and its substitutes, examination of the blood may show none of the drug in circulation within one to three hours after injection, although, since arsphenamin may be found in the urine by this method for as long as twenty-four hours and since traces of arsenic may be found in the blood for a similar or even longer periods, it is likely that traces of arsphenamin, neoarsphenamin or their oxidation products may persist in the blood for periods beyond three hours.

It cannot be stated why arsphenamin, neoarsphenamin or other organic arsenical compounds circulating in the blood for brief periods, at least, and doubtless reaching all portions of the body by arterial distribution, are deposited or retained by some organs more than others. Doubtless this phase is controlled by physical factors as solubility, surface tension, electrical charge and various chemical reactions between cells and drug and varies with different medicaments. The concentration of a drug in the cells of an organ or tissue is dependent in part at least upon its concentration in the surrounding fluids, since it appears that simple diffusion follows the law of mass action. The problem is to bring up the concentration in one organ to an efficient threshold without involving other organs and this depends primarily upon the dose and rate of elimination; small divided doses, therefore, may not produce the same effects as single large ones, but organs containing the largest amounts do not always show more effects than those containing smaller amounts. In other words, the relative concentration in which a drug is present in different organs does not determine the extent to which these are involved in its action.

The arsenic of arsphenamin and neoarsphenamin has been found largely deposited in the lungs, liver and spleen; whether this denotes that these compounds themselves or their oxidation products have an affinity for these organs cannot be stated. It is probable and possible that arsenic is found in especially large amounts in the liver, not only because there is a special affinity between this organ and arsphenamin, but because the liver is an especially active organ of elimination of arsenic in general in the bile and the arsenic is doubtless brought to it for elimination from other organs and tissues. By a quantitative colorimetric modification of Abelin's method, Kolle and Youmans[20] found arsphenamin principally in the spleen, liver, lungs, heart and kidneys of a dog three hours after intravenous injection; only traces were found in the brain and none in the cerebrospinal fluid. Clausen and Jeans have found most arsenic in the liver, spleen and kidneys after intravenous injections. Bulmer[21] found, after the intravenous injection of rabbits with organic arsenical compounds in doses corresponding to those given to man, that the arsenic is found chiefly in the liver, lungs, kidneys and long bones. The important features in their distribution were: high concentration in the liver shortly after injection; rapid reduction of this concentration, presumably by the elimination of the arsenic in the bile; the relatively large amount in the lungs, especially after large doses, and the maintenance of a high arsenic content here over a period of at least several days, and its retention by the long bones over a longer period than that shown by any other tissue analysis. Voegtlin, Smith, Dyer and Thompson[22]

have also studied the distribution of arsenic in the organs of rabbits removed 100 and 200 minutes after the intravenous injection of arsphenamin, neoarsphenamin and other arsenical compounds and found largest amounts in the bile, urine, liver, spleen and stomach contents.

As previously stated, it is probable that the large amount of arsenic found in the liver and kidneys is due to the functional activity of these organs in the elimination of arsphenamin, neoarsphenamin or the inorganic arsenites and arsenates of these compounds, rather than to any special affinity between the compounds and these organs. The high arsenic content of the lungs is probably due to the retention in the capillaries of minute emboli of precipitated arsphenamin, even though there are no clinical signs or symptoms of embolism. Likewise, the high arsenic content of the heart muscle, in contrast to the low arsenic content of the skeletal muscles, may be due to the retention in the capillaries of the heart of very minute emboli of precipitated arsphenamin swept through the lungs and distributed by the coronary arteries. At any rate, these and similar studies have shown that, after the intravenous injection of arsphenamin, neoarsphenamin and allied compounds, widespread distribution of arsenic occurs, but that storage or retention varies greatly among the different organs. The same is doubtless true after intramuscular injections, although at any one time the amount of drug in the blood is much less. As stated by Voegtlin and Thompson, solubility, molecular weight, surface tension, electric charge and dissociation are probably important factors having a bearing upon the distribution of arsenical compounds in the body.

Penetration of Arsphenamin, Neoarsphenamin and Other Organic Arsenic Compounds in the Cerebrospinal Fluid and Tissues of the Central Nervous System.—Undoubtedly, after the injection of arsphenamin and allied compounds, the drugs circulate in the blood through the brain and spinal cord, but of special interest in this connection is the question of penetration to the cerebrospinal fluid in relation to the treatment of syphilis of these organs. The subject has excited a great deal of discussion and a great deal of investigation has been devoted to quantitative studies of the cerebrospinal fluid for arsenic in the form of arsenites and arsenates. Unfortunately the methods employed have not always been acceptable for the detection of minute traces of arsenic and, more particularly, have not always adequately controlled the possibility of errors due to "normal arsenic" in the spinal fluid and tissues from food, water and other sources and of arsenic in the reagents.

It is significant that only small amounts of arsenic have been found in the brain and spinal cord. McIntosh and Fildes[23] were unable to find any at all in the brain after the intravenous injection of arsphenamin and Rudolf and Bulmer[24] have more recently stated that after the intravenous and intraspinal injection of phenarsphenamin in rabbits, arsenic could not be demonstrated in the brain or spinal cord by the Gutzeit test. McIntosh and Fildes believed that the factor governing the passage of dyes into the brain after intravenous injection was their solubility reaction. This, they state, is a peculiar and not a general lipoid one. It corresponds to a solubility in chloroform and in water or perhaps their partition coefficient in these liquids. It is probable that the same may be true of the spinal tissues owing to their similar composition and, from this standpoint, as stated by Rudolf and Bulmer, one would not expect to find arsenic in the tissues of the central nervous system. However, as shown by Voegtlin and his associates, arsenic is to be found in the brain of rabbits after the intravenous injection of arsphenamin, neoarsphenamin, sulpharsphenamin and other organic arsenical compounds, and while the results of similar studies of the tissues of the central nervous system of human beings succumbing to disease or accident during the course of arsphenamin administrations are not available, it is entirely likely that arsenic is deposited in the brain and spinal cord after the intravenous and intramuscular injection of organic arsenic compounds; furthermore, the evidences of toxic neurotropism sometimes exhibited after the administration of organic arsenic compounds, and especially those of the pentavalent type, is confirmatory evidence.

This brings up the question of whether or not the organic arsenical compounds or the arsenic of these are to be found in the cerebrospinal fluid after intravenous injection; probably no subject concerning the distribution and fate of these compounds employed in the treatment of syphilis has excited as much discussion with particular reference to the treatment of the various forms of neurosyphilis. While the exact origin of the cerebrospinal fluid is as yet unknown, it is likely that under physiological conditions this fluid is a form of secretion of the choroid plexus and ependymal cells which effectually prevent the passage of most drugs into the spinal fluid. Hexamethylenamin, uranin and chloroform may pass through and especially the former, but from the standpoint of the drugs employed in the treatment of syphilis, it may be stated that mercury has never been found in the cerebrospinal fluid except when it was injected directly into the subarachnoid space, and Catton[25] and Mehrtens[26] failed to find iodin even after intravenous injections of the iodids. In trypanosomiasis and syphilis of human beings involving the brain, it is possible that drugs may more readily pass from the blood into the spinal fluid; this is especially true in paresis, as indicated by the frequency with which antisheep hemolysin is found in the spinal fluid (Weil-Kaffka reaction), and it is almost certain that in syphilitic meningitis substances in the blood, including drugs, may find their way more readily into the cerebrospinal fluid by extravasation from the congested vessels of the meninges. This is furthermore suggested by the reports of Mehrtens and MacArthur[27] and other investigators, showing that the amount of arsenic in the cerebrospinal fluid after the intravenous injection of arsphenamin and neoarsphenamin may be increased by the injection of sterile serum which induces a mild reaction of the meninges designated as "aseptic meningitis," although it must be likewise stated in this connection that Stillman and Swift[28] and Hall, Callender and Holmbad[29] reported that the intraspinal injection of salt solution or serum did not increase the amount of arsenic in the spinal fluid after the intravenous injection of salvarsan.

It is hardly profitable here to review in detail the rather large literature bearing upon analyses of the cerebrospinal fluid for arsenic after the administration of arsphenamin and neoarsphenamin, since some investigators do not appear to have employed acceptable methods and have not sufficiently controlled the factor of normal or physiological arsenic. Sicard and Bloch[30] recovered arsenic in three out of ten cases after the intravenous injection of salvarsan, but none after intramuscular injections; Wechselmann[31] likewise found arsenic in the spinal fluid of a few cases after intravenous injections of salvarsan. Camp,[32] Ravaut[33] and Hall[34] found traces in the spinal fluids of a few cases, while Engman and his associates[35] and Citron[36] have reported negative results. Barbat[37] found arsenic in the spinal fluid in about 69 per cent. of cases when spinal puncture was conducted immediately after the intravenous injection of 0.4 gm. of salvarsan, while spinal fluids of 26 cases examined twenty-four hours later showed the presence of arsenic in all but one. Mehrtens and MacArthur[38] found arsenic in the spinal fluids of 43 per cent. of cases after the intravenous injection of 0.6 gm. arsphenamin; spinal drainage did not appear to increase the arsenic content, although intravenous injections six hours after the production of meningeal irritation resulted in the penetration of arsenic to the spinal in 92 per cent. of cases. Corbus, O'Connor, Lincoln and Gardner[39] report that the intravenous injection of hypertonic saline solution increased the amount of arsenic in the cerebrospinal fluid after the intravenous injection of arsphenamin. Cornwall and Myers,[40] in a study of spinal fluids from 151 cases of neurosyphilis treated with intravenous injections of 0.1 to 0.2 gm. of silver arsphenamin, claim that arsenic was to be found in quantitative amounts in 78 per cent. of cases two hours later and in 68 per cent. at the end of seventy-two hours.

The observations of Clausen and Jeans have proved especially interesting because they show that the penetration of arsenic into the spinal fluid is modified by syphilis and that more is to be found in cases of neurosyphilis than in normal children. These investigators also observed in several cases that the arsenic in the spinal fluid did not increase after repeated intravenous injections of arsphenamin; on the contrary, the arsenic was progressively increased.

Voegtlin and his associates found that the average arsenic content of the cerebrospinal fluid of eight normal rabbits, which were fed for several weeks on oats and kale in a special room, was 0.69 micromilligrams per gram of fluid, with a maximum of 1.55 and a minimum of 0.5 micromilligrams. These traces of arsenic represented the so-called physiological or "normal" arsenic derived from the traces of arsenic consumed with the food and drinking water. After the intravenous injection of arsphenamin (disodium salt) in dose of 0.040 gm. per kilo the maximum increase of arsenic in the spinal fluid was found about four hours later and amounted to 40 micromilligrams. At this time the blood arsenic was about 15 and the brain arsenic about 2 micromilligrams. The brain tissue of normal control rabbits contained an average of 0.02 micromilligram of "normal" or physiological arsenic and while it is difficult to properly evaluate the results insofar as penetration of arsenic into the brain tissue is concerned, since the arsenic found may be that in the blood, Voegtlin was of the opinion that the brain arsenic was actually increased since there was no parallelism between brain and blood arsenic; at any rate, there can be no doubt of the increase of spinal fluid arsenic which at this time interval was almost three times higher than the blood arsenic.

After the intravenous injection of neoarsphenamin in dose of 0.064 gm. per kilo, the arsenic content of the blood, brain and spinal fluid four hours later was about 3, 0.2 and 30 micromilligrams respectively, showing a very distinct increase of arsenic in the spinal fluid; at the end of 30 minutes the arsenic content of the spinal fluid was about 35 micromilligrams, which was the maximum in comparison with the maximum of 45 micromilligrams of arsenic 210 minutes after the intravenous injection of arsphenamin.

In a study by Fordyce, Rosen and Myers[41] of the arsenic content of the spinal fluid of 27 patients treated with intravenous injections of 0.9 gm. neoarsphenamin followed in from 24 to 30 hours by the injection of auto-serum intraspinally according to the Swift-Ellis method, it was found that 88.5 per cent. of the spinal fluids showed the presence of arsenic at the time of at least one examination, varying in amount from a trace of 83.6 mg. of metallic arsenic per 100 grams of dried specimen.

In a similar study[42] of 63 patients suffering with various types of neurosyphilis, many in an advanced stage of the disease, and treated with intravenous injections of 0.4 gm. arsphenamin followed by the intraspinal injection of auto-serum, a total of 257 spinal fluids were examined at intervals varying from 30 minutes to 4 months; of these 257 fluids, 88 per cent. showed the presence of arsenic at the time of at least one examination, varying from a trace to 73 mg. per 100 gm. of dried specimen.

In an additional study[43] of 72 spinal fluids from 40 patients with syphilis in various stages of the disease treated with intravenous injections of 0.4 gm. arsphenamin followed by withdrawal of 20–40 c.c. of spinal fluid at varying intervals, Fordyce and his associates found no arsenic in 17 per cent. of the fluids, while traces to 54.3 mg. per 100 gm. of dried fluid were found in 83 per cent. of fluids.

These very extensive investigations have shown, therefore, that after a series of intravenous injections of arsphenamin, some arsenic is to be found in the spinal fluids of over 80 per cent. of cases of syphilis at least some one time; the number showing arsenic is slightly increased, however, when patients are treated in addition with an intraspinal injection of auto-serum removed from 50 c.c. of blood drawn after the intravenous injection of arsphenamin or neoarsphenamin.

Voegtlin and his associates found that sulpharsphenamin injected intravenously was more active in the destruction of trypanosomes placed in the subarachnoid space of the cerebral meninges than either arsphenamin or neoarsphenamin; yet the amounts of arsenic to be found in the brain tissue and cerebrospinal fluid after the intravenous injection of sulpharsphenamin was much less. In these experiments sulpharsphenamin, tryparsamid and 3-amino-4-oxyphenyl arsonic acid proved of most therapeutic power, although, as previously stated, the degree of penetrative power did not bear a relation to the amounts of inorganic arsenic recovered from the brain and cerebrospinal fluid six hours after the intravenous injections; it may be quite possible that arsenic penetrates at a later period in larger amounts. Neoarsphenamin proved slightly more efficient than arsphenamin and both of these were more efficient than silver arsphenamin. Curiously, the aromatic pentavalent arsenical compounds, as tryparsamid, arsacetin and atoxyl, appeared to possess greater therapeutic activity in these experiments than these well-known arsphenamins and sulpharsphenamin proved the most effective of the arsenobenzene derivatives studied.

Furthermore it cannot be definitely stated just how far arsphenamin and neoarsphenamin contained in serum or saline solution and injected into the subarachnoid space of the spinal cord, are distributed. The circulation of the spinal fluid appears to be very slow. According to Cavazzani[44] and Hill,[45] substances injected into the subarachnoid space of the cord require from twenty minutes to two hours for general distribution. The direction of circulation appears to be from the ventricles outward and downward; an active circulation toward the ventricles has not been demonstrated, although substances injected intraspinally may become widely diffused in the subarachnoid space. Dandy and Blackfan[46]

have shown, for example, that, after intraspinal injections of finely divided carbon, there was rapid distribution of the particles throughout the subarachnoid space. They showed also that phenolsulphonphthalein injected intraspinally was demonstrable in fluid obtained a few minutes later from the ventricles; these observations naturally possess a bearing upon the distribution of drugs injected intraspinally and indicate that arsphenamin or neoarsphenamin injected intraspinally very probably diffuse upward with wide distribution in the subarachnoid space of both cord and cerebrum.

Elimination of Arsphenamin, Neoarsphenamin, Tryparsamid, and Allied Drugs.—Before discussing the relation of distribution and storage of arsenic in the different tissues following the administration of arsphenamin, neoarsphenamin and allied drugs in relation to the treatment of syphilis, it is first necessary to review briefly our information regarding the elimination of these compounds after intravenous and intramuscular injections.

Elimination of Arsphenamin and Allied Drugs from the Blood.—In the first place, how long does arsphenamin and its allied drugs remain in the blood after the intravenous injection of the usual amounts (0.3–0. 6 gm.) employed in the treatment of syphilis? Tests for salvarsan itself made by the Abelin method have shown very rapid elimination. Thus Treupel[47] found that, after the intravenous injection of 0.4 gm. salvarsan dissolved in water, the average time of disappearance from the blood was thirteen and one-half hours, whereas after the injection of the same amount dissolved in serum it disappeared in twelve hours. Similar results were observed by Spiethoff[48] and the results interpreted as indicating that salvarsan dissolved in serum was less toxic, more rapidly removed from the blood and stored in the organs with consequently more time for elimination.

Robertson and Klauder[49] were unable to find any salvarsan in blood removed ninety minutes after intravenous injections, although positive reactions were observed after ten and fifteen minutes. Schreus and Holländer,[50] applying the same test, were never able to detect salvarsan in the blood serum more than three hours after injection. Fifteen minutes after injection, only about 30 per cent. of the salvarsan was found in the blood and only 20 per cent. at the end of thirty minutes. Neither the dose (0.3 to 0.6 gm.) nor the number of injections had any influence upon the duration of salvarsan in the blood, which argues against a cumulative effect. However, since salvarsan has been detected in the urine by Abelin's method for 24 hours after intravenous injection, it is possible that small amounts may circulate for that length of time at least.

The elimination of arsphenamin and neoarsphenamin from the blood has also been studied by means of quantitative arsenic determinations. Barbat and Myers found that, after the intravenous injection of arsphenamin or neoarsphenamin, only about 25 per cent. could be recovered as arsenic one-half hour later. Clausen and Jeans, employing a modification of Gutzeit's method for quantitative arsenic determinations, found that little or no arsenic was present in the corpuscles after the intravenous injection of arsphenamin but that all was present in the plasma or serum. These investigators also observed that, after the intravenous injection of children with about 0.010 gm. of arsphenamin per kilogram of weight, arsenic rapidly disappears from the circulation, only 50 per cent. remaining for ten minutes and only 10 per cent. at the end of one hour. After the first hour, the rate of disappearance of arsenic was much slower and remained almost constant for six hours.

Webster[51] found ½ to ⅓ mg. of arsenic per 100 c.c. of blood one hour after the intravenous injection of 0.6 gm. arsphenamin; after the administration of 0.9 gm. neoarsphenamin, ⅕ mg. of arsenic per 100 c.c. of blood was found twenty-four hours later, and $\frac{1}{10}$ mg. and $\frac{1}{40}$ mg. after 2 and 5 days respectively. Young[52] in an interesting experimental study, found that, if the serum of goats previously injected with arsphenamin is dialyzed, some of the arsenic is removed, but that the balance is bound to the serum and cannot be dialyzed nor give the usual qualitative reactions. Young found that the inorganic arsenic compounds did not behave in this manner and concluded that it is the arsenic which is bound in the blood and tissues that is slowly eliminated in the urine and feces.

Fordyce, Rosen and Myers[53] have determined the arsenic content of the blood of syphilitic and a few non-syphilitic persons at varying intervals after the intravenous injection of arsphenamin, silver arsphenamin, neoarsphenamin and tryparsamid; *immediately* after injection as much as 37.9 to 66.1 per cent. of arsenic had already disappeared from the blood. During the first twenty-four hours there was observed a gradual lowering of the arsenic equilibrium in the blood after the injection of both arsphenamin and silver

arsphenamin, followed by a marked increase with a gradual diminution at later intervals. Abnormal values, both high and low, for any given interval (partition coefficient) were met with in certain individuals with an arsenic idiosyncrasy or predisposition to intoxication by the drug and rapid disappearance of the arsenic from the blood may be a warning, in the opinion of Fordyce and his associates, of latent arsenical intoxications. In other words, the rate of excretion and retention of arsenic after intravenous injections of arsphenamin is irregular with two well-defined groups which may be classified as "short period" and "long period;" this renders difficult the selection of the most suitable interval for obtaining arsphenaminized serum for intraspinal therapy. Similar results have been reported by Wechselmann and his associates.[54]

I have studied this question of elimination of arsphenamin and neoarsphenamin from the blood by applying a trypanocidal test and the results have proved very interesting and especially useful, since they indicate the duration of the therapeutically active arsphenamin or oxidation products, which after all is of most practical interest in relation to the treatment of syphilis. These tests were conducted by injecting a series of white rats intravenously with 0.005 gm. arsphenamin (disodium salt) and 0.010 gm. neoarsphenamin corresponding respectively to 0.3 and 0.6 gm. per 60 kilo and subsequently infecting animals of both series by intraperitoneal injections of 500,000 Tr. equiperdum 1, 2, 3, 4, 5, 6, 24, 48, 72 and 96 hours later. Rats infected one to six hours after intravenous injections of both drugs usually remained sterile and lived indefinitely, while untreated controls died on the fifth day; animals infected twenty-four to ninety-six hours and longer after intravenous injections of both drugs in the doses mentioned invariably developed trypanosomiasis and died on the fifth to seventh day. These experiments have indicated, therefore, that both arsphenamin and neoarsphenamin and their therapeutically active oxids are rapidly removed from the blood, although, of course, when larger doses were injected, the effects were prolonged for periods slightly greater than just described.

Elimination of Arsphenamin and Neoarsphenamin in the Urine.—A large number of investigations have been devoted to a study of the elimination of arsphenamin and allied compounds as such in the urine and more especially to the elimination of arsenic in the urine and feces after the administration of these drugs. These determinations have had great interest and value in yielding information regarding the fate of these medicaments and the route and rate of their excretion; they have also yielded information bearing upon the mechanism of their therapeutic activity as well as upon the toxic effects sometimes following their administration.

In 1911 Abelin developed a method for the detection of arsphenamin itself in the urine after intramuscular and intravenous injections. This diazo color reaction is merely an indication of the presence of an aromatic amino group, but is regarded by Abelin as specific for the presence of the R-As = As-R radical. In the examination of the urine of thirty-four cases of syphilis, he found that arsphenamin could be demonstrated within five to fifteen minutes after an intravenous injection with negative reactions usually after five or six hours thereafter. In his opinion the rapidity of excretion of arsphenamin and allied drugs is related to the patient's general condition, although he found excretion irregular in that, when the same dose is given several individuals, a marked reaction occurs in some with weak or negative reactions in others.

Leredde and Rubinstein[55] have applied the test to a series of 435 patients receiving 0.15 to 1.2 gm. neoarsphenamin intravenously and in eight of these elimination was delayed, all of the latter being in the chronic stages of syphilis. In their experience elimination usually began in five minutes and was completed in twenty-four hours, the degree and duration of elimination being proportional to the amount injected. They further ascertained that in patients who had received eight, ten and fifteen injections in a course, elimination remained normal.

Escallon,[56] Rieber,[57] Koetter,[58] Authenried and Taege[59] and others have reported more or less favorably upon this test as a means for determining the rate of elimination of arsphenamin, neoarsphenamin and allied drugs. The majority have observed that elimination begins within the first hour after intravenous injections and that it is completed within twenty-four hours. Koetter found that, after the intravenous injection of 0.4 gm. arsphenamin dissolved in water, the drug disappeared from the urine in nineteen hours, whereas when dissolved in the patient's serum it was more slowly eliminated, being found present for forty-two hours. Similar results were reported by Bergmann[60] on the basis of arsenic determinations and the results have been interpreted as showing that arsphenamin and neoarsphenamin dissolved in serum are less toxic, less rapidly eliminated and consequently therapeutically active for longer periods.

Recently, Beeson and Albrecht[61] have reported favorably upon the Abelin reaction applied to urine and believe that it possesses real value as a simple and sensitive test for arsphenamin and neoarsphenamin. In their experience it is specific for these and allied drugs and other substances of the benzene series, as acetylsalicylic acid, acetphenetidin, antipyrin and the salicylates were found not to give the reaction either directly in the test-tube or in the urine after administration. Urine from patients with pneumonia, nephritis, rheumatism and gonorrhea were also tested and found to yield negative reactions. By a slight ring modification of the Abelin test they have been able to detect arsphenamin in the test-tube in amounts as small as 0.0001 to 0.0003 gm. in 10 c.c. of water and neoarsphenamin in amounts as small as 0.0001 to 0.0002 gm. in 10 c.c. of urine. After the examination of the urine of fifty cases of syphilis treated with intravenous injections of neoarsphenamin in concentrated solutions, they concluded that the test was not only specific and very sensitive but of some practical value. In their experience elimination was somewhat slower than reported by Abelin, but usually complete in twenty-four hours; in cases where elimination is greatly retarded or does not occur at all, they believe that a careful examination of the patient is required before treatment is continued.

My experience with this test has not been satisfactory. When arsphenamin or neoarsphenamin were added to water or normal urine, satisfactory reactions were observed even with the small amounts mentioned by Beeson and Albrecht; but tests of the urine collected at intervals of 1, 2, 3, 4 and 24 hours after intravenous injections of 0.6 gm. arsphenamin or 0.9 gm. neoarsphenamin have generally yielded uncertain and unsatisfactory reactions. Sometimes a sufficiently certain and clear reaction was observed with one-hour urine, but after that the usual yellowish-brown color yielded by all specimens, irrespective of the administration of the drugs, interfered with the detection of the color reaction supposed to be produced by the eliminated drugs. I can readily enough understand that a test of this sort for determining the eliminative powers of the kidneys may prove of considerable value and especially with cases presenting toxicological effects, as a means for controlling dosage and indicating extra care in treatment of elimination were found to be below a normal average, but in my experience the Abelin reaction and its modifications have failed to yield reactions and information of this sort in actual practice.

Elimination of Arsenic in Urine, Bile and Feces, after the Administration of Arsphenamin, Neoarsphenamin and Allied Drugs.—The majority of investigators, however, have studied the elimination of arsphenamin and its substitutes by quantitative arsenic determinations, since it is well known that arsenic may be eliminated in the urine and feces for days and weeks after the Abelin test has yielded negative reactions. Sieburg,[62] for example, found that none or only a very small part of the arsphenamin is excreted as such in the urine, pentavalent organic arsenical compounds and inorganic arsenites being usually recovered. The literature up to 1913 has been thoroughly reviewed by Ullman;[63] unfortunately, however, many of the earlier investigations possess but little value or interest because of faulty methods employed for the destruction of the organic matter, for the quantitative determination of arsenic and failure to check adequately the "normal" or physiological arsenic that may be present due to absorption from food, drugs, etc.

Ullman, however, has made a limited number of daily analyses of the urine and feces of patients receiving intravenous injections of arsphenamin, using the hydrochloric acid potassium chlorate method for destroying organic matter and determining arsenic by the Marsh mirror method after converting it into a sulphid. His check analyses indicate that 26 per cent. of the arsenic was lost in the process of destruction of organic matter. In the urine of the first of his series of patients, who received 0.4 gm. arsphenamin intravenously, he detected during the first three days 3.9, 3.1 and 1.4 mg. arsenic, respectively. This is equivalent to a total of 7 per cent. of the amount of arsenic administered. With doses of 0.3 gm. arsphenamin he found in one patient 4.6, 2.2 and 1.6 mg. arsenic, respectively, during the first three days, and in another patient 8.2, 2.7, 0.3, 0.8 and 0.1 mg., respectively, during the first five days. The percentages of elimination during the first three

days were 9.3 and 12.2 per cent., respectively. Since then detailed studies have been published by Frenkel-Herden and Navassart,[64] Wilcox and Webster,[65] Lockemann,[66] Bergman,[67] Weiss and Raiziss,[68] Clausen and Jeans,[69] Bernstein,[70] Fordyce, Rosen and Myers[71] and others but with varying results largely because of the different methods employed. For example, Weiss and Raiziss, employing a modification of the Green iodometric micro-titration method, studied elimination of arsenic in three cases of chronic syphilis with optic atrophy after the intravenous injection of 0.6 gm. of alkalized arsphenamin dissolved in 120 c.c. of distilled water and given twice weekly, weekly or fortnightly. Very small quantities of arsenic, detectable only by very delicate methods of analysis, were found in the daily urine. Thus, after a dose of 0.6 gm. containing from 187 to 188.6 mg. arsenic, from 3 to 8.8 per cent. was eliminated in three days and from 4.9 to 13.5 per cent. in the course of a fortnight. By far the greatest amount of arsenic eliminated by the kidneys was, therefore, found in the urine of the first three days after injection. During the succeeding days, the amounts gradually declined, until by the fourteenth day they usually fell to a level of from 0.1 to 0.5 mg. which approaches the limit of accuracy of the method used. Only a small part of the arsenic derived from arsphenamin was eliminated, therefore, by their three patients through the urine, 13.5 per cent. being the highest amount in fourteen days. The elimination was larger after the second and third doses than after the first. But using constant doses of 0.6 gm. arsphenamin, there was considerable variation in the amounts of arsenic eliminated during a given interval of time by any one patient as well as by the three different patients presumably in the same chronic stage of syphilis and treated under the same conditions

Clausen and Jeans employing a modified Gutzeit method, have made arsenic determinations of the urine during ½, 1, 3, 12 and 24 hour intervals following the intravenous administration of arsphenamin (disodium salt) to children in dose of 0.010 gm. per kilo; also of daily total specimens thereafter for two weeks or more. The rate of excretion was at first very high, as would be expected from the high arsenic content of the blood at this time. After dropping to a minimum at 20 to 60 hours, the rate of excretion in all cases but one rose to a maximum at 55 to 132 hours, after which the level steadily fell.

Bernstein concluded from his study that, while the arsenic elimination after the injection of sulfoxylate salvarsan is high during the first six hours, yet as a whole the compound is more slowly oxidized than silver salvarsan and neosalvarsan and thereby longer retained with more prolonged therapeutic effects. Lockemann[72] had previously shown that neosalvarsan was more rapidly eliminated than arsphenmin, but recently Galonnier[73] reported that after intravenous and intramuscular injections of sulfarsenol and neoarsphenamin the elimination curves were quite similar. After the intravenous injection of sulfarsenol, arsenic was found in the urine in one hour; after the injection of neoarsphenamin, arsenic was found in ten minutes; but both reached the maximum of excretion at the end of the first day. After intramuscular injections, the maximum excretion of arsenic was at the end of the second day for both compounds.

It was soon discovered by the Navassarts[74] and others that large amounts of arsenic were to be found in the *feces* after the administration of arsphenamin and neoarsphenamin and at first this was ascribed to elimination through the intestinal wall until Obregia and Carniol[75] showed that arsenic was actively eliminated through the bile, being found as early as twenty-five minutes after the intravenous injection of neoarsphenamin. Pomaret[76] also found 10 mg. of arsenic in 5 c.c. of dog's bile collected one hour after the intravenous injection of 0.3 gm. neoarsphenamin.

Subsequent investigations have shown that from three to five times more arsenic is to be found in the feces than in the urine; Clausen and Jeans, for example, have found five times more in the feces than in the urine of children injected intravenously with 0.010 gm. arsphenamin (disodium salt) per kilo of weight. Recently Underhill and Davis[77] have likewise found that, after the intravenous injection of arsphenamin and neoarsphenamin, the total percentage of arsenic excreted in the feces is larger than in the urine, being as high as 53.76 per cent. in one week. They observed that arsenic appeared in the urine within a few hours after intravenous injections, but more slowly in the feces (three or four days), the maximum excretion occurring in the urine on the same or following day. In a series of injections, the arsenic excretion was found small in the first interval in both urine and feces, but increased after each succeeding injection. This is due to the larger amount of arsenic stored in the liver after the administration of all arsenic compounds including the organic trivalent and pentavalent compounds, with elimination through the bile.

In addition to elimination through the bile, some arsenic is apparently excreted by the *intestinal mucosa*. Analyses have shown the presence of arsenic in these tissues and it is well known that arsenic may produce histological changes in the epithelium composed largely of cloudy swelling and fatty degeneration along with hyperemia of the submucosa. Of course, these changes may be due to the absorption of arsenic from the bile, but at present pharmacologists are in general of the opinion that these effects are due to elimination.

Up to the present time, however, elimination through the *bile* has been studied exclusively on animals, although the readiness with which bile can now be obtained from human

subjects by the Meltzer-Lyon method opens up a means for the study of elimination by this route and it is possible that future investigations will develop a practical means for determining the elimination of arsenic from the kidneys and liver after the first dose or two of arsphenamin, neoarsphenamin or allied compounds employed in the treatment of chronic syphilis as a means for preventing such accidents as icterus and dermatitis which may be caused by faulty elimination.

As will be shortly discussed with more detail, the investigations of Voegtlin and his associates have shown that the excretion of arsenic varies considerably in different normal rats after the administration of any one compound and that elimination after the injection of the different organic arsenicals varies greatly according to their chemical constitution as affecting their physical properties. Arsphenamin, for example, is largely eliminated by the liver in the bile; neoarsphenamin and atoxyl, by the kidneys in the urine; etc. Ligation of the common bile duct in aminals increased, therefore, the toxicity of arsphenamin but not of atoxyl and only slightly of neoarsphenamin.

Elimination of Arsenic in Perspiration, Skin, Vomitus, Saliva and Milk.— In 1912, Ullman[78] found a questionable trace of arsenic in the *perspiration* of a patient after the intravenous injection of arsphenamin, and recently Fordyce and his associates[79] have greatly renewed interest in the presence of arsenic in the skin. As stated by these authors, arsenic is known to have a marked affinity for epithelial cells and in chronic poisoning may produce a wide variety of cutaneous lesions. It is possible that dermatitis exfoliativa may have this origin and especially if there is present a coexisting peripheral neuritis; in their opinion, when normal elimination of arsenic through the skin is interfered with, its deposition in the cutaneous layers is sufficient to produce irritation and consequent edema which interferes with the functional activity of the skin and results in the production of dermatitis. Analysis of scales from 22 cases of syphilis, developing desquamating rashes after the administration of arsenical compounds, showed the presence of arsenic in 20, the amounts varying from 0.123 to 315.9 mg. Six batches were from non-syphilitic cases and showed from a trace to 1.63 mg. of arsenic; three of these cases were known to have received arsenious acid or cacodylate of soda.

Obregia and Carniol, previously mentioned, have demonstrated the elimination of arsenic in *saliva* after the administration of neoarsphenamin and this observation is of interest in connection with the well known clinical observation that the majority of patients complain of ether taste during the administration of neoarsphenamin and allied drugs.

It is also of interest that Weiss and Raiziss observed arsenic in the *vomitus* of one case four hours after the intravenous injection of arsphenamin; this may have been due to regurgitation of bile from the duodenum or elimination by the gastric mucosa.

It is well known that a syphilitic child, nursing its mother while the latter is under treatment with arsphenamin or neoarsphenamin, shows clinical effects; indeed, this method of treating the syphilitic infant is of considerable practical value, as I shall discuss in a later chapter. Undoubtedly the drugs must be excreted in the *milk* in a therapeutically active state in at least small amounts and analyses of the milk of lactating women under treatment with arsphenamin and neoarsphenamin have usually shown the presence of arsenic.[80] The literature is reviewed by Fordyce, Rosen and Myers[81] who likewise analyzed the milk of fifteen women for "normal" arsenic with generally negative results. They also examined the milk of twelve women after the intravenous injection of 0.2 to 0.3 gm. arsphenamin and concluded that a nursling probably receives sufficient arsenic in its daily feedings to be of some therapeutic value. Therefore, the energetic treatment of syphilitic mothers during lactation is advisable in order that the infant may have the benefit of this simple method of administration in addition to the other forms of treatment.

Variations in the Rate of Elimination of Arsenic.—As shown by Voegtlin and Thompson,[82] the rate of excretion of arsenic by different rats, following the intravenous injection of various organic arsenical compounds, has varied considerably and in their opinion this variation in the rate of excretion in different individuals is one of the important factors regulating toxicity and therapeutic activity. According to their results, variations in the rate of excretion of arsenic may occur not only in cases of impaired kidney function but also in cases of normal renal function, which may account for some of the toxic reactions sometimes observed in human subjects presenting no manifestations of renal disease. In this connection it may be stated that they observed greater variations in the rate of excretion of the arsenic of neoarsphenamin than in the case of arsphenamin, and, as I shall discuss in Chapter XLVI, it is significant that the arsenic of the former is more irritating to the kidneys of rats than the arsenic of the latter.

The directly toxic trivalent oxid compounds, as "arsenoxid," showed the slowest rate of excretion, and these are the drugs most toxic for both host and parasites, although in their experiments there was no direct relation between rate of elimination, toxicity and trypanocidal activity due, probably, to differences in the physical properties of the compounds with special reference to their solubility in the body fluids.

The pentavalent arsenical compounds with the exception of arsenic acid, were eliminated most rapidly, while the arsenobenzene compounds occupied an intermediate position. The ratio between urinary and fecal excretion of the arsenic of these different compounds also showed great variations in otherwise normal rats. There is no reason to doubt but that similar conditions exist among human beings and probably even to a greater degree, since elimination by both the kidneys and bile may be still further reduced or altered as a result of syphilis or previous medication.

Variations in the Physical and Chemical Constitution of Arsenical Compounds in Relation to Elimination of Arsenic.—According to Voegtlin and Thompson the rate of excretion of arsenic appears to bear a relation to the chemical constitution of the arsenical insofar as this determines physical properties. The rate of excretion, toxicity and therapeutic activity were found not to bear a direct relation to each other in the case of the trivalent oxids, which lack of correlation they have ascribed as probably due to differences in physical properties, particularly the solubility of these compounds. In addition, molecular weight, surface tension, electric charge and dissociation are mentioned as probable important factors having a bearing on the route and rate of excretion, although information relating to the physical properties of the compounds studied by them was very meager. The arsenic of arsphenamin was found to be largely excreted by the liver, whereas that of the corresponding oxid was excreted mainly by the kidneys which is probably due to a difference in the distribution of these compounds in the body as previously discussed, since the same physical factors as modified by chemical constitution are believed to modify the distribution of the organic arsenical compounds.

Retention and Elimination of Arsphenamin, Neoarsphenamin and Allied Drugs in Relation to the Treatment of Syphilis.—And now it is profitable to inquire what relation the results of these investigations upon the retention and elimination of arsphenamin, neoarsphenamin and allied drugs bear to the treatment of syphilis, especially the retention and elimination of the pentavalent organic arsenic and inorganic arsenic derivatives of these compounds. Insofar as retention is concerned, it may be stated that our main interest is in the question whether or not the retained arsenic is therapeutically active; the

question of elimination is of chief interest in relation to the toxicological effects produced by deficient excretion and stored arsenic.

In the first place, it is apparent that arsphenamin, neoarsphenamin and their therapeutically active "oxids" are rather quickly eliminated from the blood and that, indeed, these drugs may be eliminated in part at least in the urine and bile unchanged. From this we may conclude that only a portion of these drugs injected intravenously exert curative effects in the blood, lymph and tissues, owing to the rapidity of elimination; after intramuscular and subcutaneous injections, elimination is of course much slower and for this reason it is highly desirable to have at our command a therapeutically active arsenical compound that may be repeatedly administered by these routes in adequate amounts. It does not appear that the *amount* of these drugs administered at one time has any important relation to rapidity of elimination, that is to say, after the injection of small doses, as 0.1 gm. to an adult, a portion is rapidly eliminated as well as after the injection of 0.6 gm., although, of course, more is eliminated in the urine, bile and by other channels after the latter dose than after the former. But there does not appear to be a constant threshold; that is, it does not appear that after a certain small dose all of the drug is retained and utilized and that elimination of the therapeutically active drug occurs only when this amount is exceeded. This point is not without practical significance, because, if it were the case that all of certain small doses of arsphenamin and neoarsphenamin were utilized, it would readily become a principle of treatment not to give more than this dose but to repeat the administrations at such intervals as would maintain this threshold over a sufficient period of time for therapeutic results. As it stands, however, the evidence of investigations summarized above shows that elimination of the active drugs begins promptly after small as well as after large doses and a study of the rate and amount of elimination has not greatly aided us in arriving at conclusions regarding the amount of drug to administer at one time or the frequency of administrations. More helpful data has been obtained by trypanocidal and spirocheticidal tests in infections of the lower animals, as discussed in Chapters XVIII and XXIX. Approximately 50 per cent. of the arsenic of the trivalent and pentavalent organic arsenical compounds employed in the treatment of syphilis is retained by the tissues and slowly eliminated.

Therapeutic Activity of Stored Arsenic.—The question arises regarding the therapeutic activity of this stored arsenic. Of course, it is reasonable to believe that any of these compounds stored in the form of trivalent compounds may be converted into the therapeutically active "oxids" and exert spirocheticidal effects, but it is not known just how long these compounds exist before they are changed into less active substances and I believe that a portion at least of the stored arsenic is in the form of the therapeutically inert pentavalent compounds. Several European authors have laid stress upon the formation of "depots" of drug for slow absorption and prolonged therapeutic effects; I believe in this idea insofar as it refers to the storage of therapeutically active forms of drug as in intramuscular injections, but, as stated above, it is very doubtful if all of the arsenic which we find stored in the liver, spleen and other organs is therapeutically active. Without much doubt, some of this arsenic is re-absorbed into the blood, as indicated by the results of analyses over prolonged periods of time, but does it do any good before final elimination in the excreta? Wechselmann, Lockemann and Ulrich[82] have recently expressed the opinion that clinical observations do not indicate that this arsenic is therapeutically active and that the maximum of spirocheticidal

effects is accomplished during the comparatively short time in which large amounts of active forms of the drug circulate in the blood.

My own experiments have likewise indicated that the arsenic which is stored in the liver of rabbits after repeated intravenous injections of arsphenamin and neoarsphenamin is not therapeutically active. I have injected rabbits intravenously once a week with 0.030 gm. of arsphenamin (disodium salt) for six weeks and then tested emulsions of the liver in saline solution for trypanocidal effects by injecting large amounts into the peritoneal cavities of rats infected with *Tr. equiperdum,* with negative results. In one of these experiments, 100 grams of liver from several treated rabbits was thoroughly macerated and autolyzed under sterile conditions in 200 c.c. of sterile water. After centrifuging briefly to remove the heavier particles, the supernatant fluid was found to contain 0.00035 gm. of arsenic per cubic centimeter. From 1 to 10 c.c. of this fluid, carrying from 0.00035 to 0.0035 gm. of arsenic, was injected into a series of infected rats per kilo of weight with absolutely no influence upon the development and course of the resulting acute trypanosomiasis; untreated controls died on the sixth day after inoculation and controls treated at the same time with intraperitoneal injections of 0.003 gm. of arsphenamin (disodium salt) per kilo lived indefinitely.

Of course, experiments of this kind are not conclusive, but there is nothing to indicate that all of stored arsenic re-absorbed into the blood is again rendered therapeutically active, although it is common clinical experience to observe that after a course of intravenous injections of arsphenamin or neoarsphenamin followed by periods of rest of several months duration, the Wassermann reaction becomes weaker or negative along with clinical improvement. Such occurrences naturally suggest that the stored arsenic has continued to function therapeutically as is probably true of that oxidized into the "arsenoxid" compounds, but that which is further oxidized into pentavalent arsenic is not, unless it aids in stimulating antibody production; the effects upon the Wassermann reaction at least may be due to a continued elimination of the "reagin" from the blood rather than to a further destruction of spirochetes.

Naturally, anything interfering with the rate of elimination of the therapeutically active forms of the organic arsenical compounds may be expected to increase parasiticidal effects. Indeed, as previously mentioned, Voegtlin and Thompson have shown in trypanocidal tests with rats that there is an important relation between rate of excretion on the one hand, and toxicity and parasiticidal activity on the other. This relationship appeared to depend upon the differences in physical properties of the trivalent and pentavalent arsenicals studied. As pointed out by them, changes in the chemical constitution of these compounds affect the physical properties, which in turn determine the rate of diffusion and distribution in the body as well as the route and rate of excretion of the arsenic, thus governing the toxicity for the host and parasites alike. In confirmation of these observations, Voegtlin, Dyer and Miller[83] found that ligation of both ureters increased the toxicity and trypanocidal activity of those arsenical compounds which normally show a rapid rate of urinary excretion, as neoarsphenamin and sodium arsenate. The toxicity and trypanocidal activity were not increased by this procedure, however, for these compounds as "arsenoxid," sodium arsenate and arsphenamin have a low rate of urinary excretion. Complete obstruction of the bile duct increased the parasiticidal action of arsphenamin and neoarsphenamin, but not of atoxyl, and produced only slight changes in the toxicity of neoarsphenamin. The negative results observed with atoxyl were expected, in view of the fact that this compound is eliminated very rapidly by the kidneys;

it was surprising, however, that ligation of bile duct did not increase the toxicity of arsphenamin and neoarsphenamin to a greater extent, which probably was due to a compensatory function of the liver.

While a portion of the stored arsenic may not be therapeutically active or capable of being rendered so after re-absorption into the blood and distribution, there can be no doubt but that it may exert injurious effects. The "arsenoxid" itself may injure the tissues of the host, because toxicity tests on rats have shown that it is at least five to eight times more toxic than arsphenamin but the tissues apparently are able to continue oxidation of this compound to the less toxic pentavalent arsenic which the parasites are not able to do, at least not to the same extent. In this way Voegtlin has explained the toxicity of "arsenoxid" for parasites (parasitropism), without apparent injury of the host (organotropism). But aside from this, I believe that the therapeutically inactive pentavalent arsenic may be injurious to the tissues and anything which hinders its elimination does not benefit the syphilitic therapeutically but may cause injury by toxic effects.

Effects of Arsphenamin and Neoarsphenamin upon Blood Pressure.—In 1915 Rolleston[84] studied the effects of intravenous injections of 0.6 to 0.9 gm. neoarsphenamin upon the blood pressures of one hundred consecutive cases of syphilis, almost all in the secondary stage, and with an average age of 24 years. The pressure (systolic and diastolic) was taken (1) on one or more days before the injection was given, (2) about seven hours after the injection, and (3) on three or sometimes four subsequent mornings. In 19 of these cases the blood-pressure was also taken during the intravenous injection of neoarsphenamin. Included in the 100 cases were 12 cases in which the blood-pressure was investigated in connection with both first and second injections of neoarsphenamin usually at a month's interval. Mercer's sphygmomanometer, with an arm-cuff of 14 cm. was used. The auscultatory method was employed, the maximum systolic pressure being estimated as the mean between the points where the auscultatory sound disappeared and reappeared, and the diastolic pressure at the time of the so-called fourth phase, where the intensity of the auscultatory sound suddenly diminishes. Rolleston took all the blood-pressure estimations himself so that errors from more than one personal equation were avoided. As a result of this thorough study, the following conclusions were reached:

1. The average of both the systolic and diastolic blood-pressures on the days after injection was generally lower than before injection, the fall in the systolic being slightly more marked than in the diastolic pressure. This fall, however, was very probably not a direct result of the action of neoarsphenamin, and, in part at least, may have been due to rest in bed.

2. The blood-pressure seven hours after the intravenous injection of neoarsphenamin was usually lower than it was before. The fall in the diastolic pressure was slightly less than that of the systolic pressure. The fall could not be explained as due to fever.

3. As compared with the blood-pressure taken seven hours after the intravenous injection of neoarsphenamin, the average of the blood-pressure estimations on subsequent days was usually lower. This was more marked in the diastolic than in the systolic blood-pressure.

4. During the actual intravenous injection of neoarsphenamin both the systolic and diastolic pressures were nearly always higher than on other occasions. This appeared to be due to excitement. During the operation the pressures varied considerably, the systolic blood-pressure being more affected than the diastolic.

5. The blood-pressure before the first injection of neoarsphenamin was nearly always higher than the blood-pressure before the second injection of neoarsphenamin, but here again mental excitement may have been responsible.

6. Finally, the general effect of intravenous injections of neoarsphenamin was rather to lower, certainly not to increase, the arterial blood-pressure.

In 1918, Jackson and Smith,[85] in a study of the mechanism of the immediate toxic reactions sometimes following the intravenous administration of

arsphenamin, found that the slow injection of dilute alkaline solutions of arsphenamin (monosodium salt) into anesthetized dogs in doses similar to those administered human beings in the treatment of syphilis, produced no striking results. However, as the rate of injection and the concentration of the drug were increased, symptoms of dilatation of the heart were apparent, perhaps mainly of the right side at first, along with a progressive increase of pulmonary blood-pressure, and a slow, gradual, but not severe, fall of the systemic pressure. Smith[86] subsequently found similar effects produced by the intravenous injection of "arsenoxid." Jackson and Smith, and subsequently Jackson and Raap,[87] found that this increase in pulmonary blood-pressure was not due to contraction of the musculature of the bronchioles, but probably to the effects of obstruction of the pulmonary circulation, due partly to the alkalinity of the solutions, possibly to the presence of embolic precipitates in the vessels, and partly to the specific action of the drug itself on the musculature of the arterioles.

Similar studies do not appear to have been made with neoarsphenamin, but, since solutions of this drug are approximately neutral, do not produce "nitritoid crises" as frequently as arsphenamin and are very much less likely to produce intravascular agglutination of erythrocytes and serum precipitates, I believe that it is highly probable that similar effects upon the pulmonary blood pressure are not produced.

In brief, therefore, it may be stated that the slow intravenous injection of 0.6 gm. arsphenamin in alkaline solution, well diluted in at least 100 to 150 c.c. of water, does not produce any noticeable effects upon the general systemic or pulmonary blood-pressures; certainly there is no increase of general systemic blood-pressures produced by neoarsphenamin, as shown by Rolleston. When arsphenamin is injected in acid solutions, which produce marked agglutination of erythrocytes and heavy precipitates in the blood, embolism occurs in the lungs with a greatly increased blood pressure; apparently no effect, however, is exerted upon the musculature of the bronchioles which speaks against an anaphylactic basis of the acute clinical reaction partly caused by this pulmonary embolism. It may be that the alkali and the drug itself also cause some muscular constriction of the pulmonary vessels and thereby increase pulmonary pressure with or without the embolism. This results in increased work for the heart which may dilate or pass into delirium cordis, but, since these are toxicological effects, I shall discuss the subject with more detail in a succeeding chapter concerning the "nitritoid crisis" and other arsphenamin reactions. Suffice to state that in ordinary doses properly administered to human beings, the pharmacological effects of arsphenamin and neoarsphenamin upon the general and pulmonary blood-pressures are slight or altogether without discernible alteration except for the effects that may be produced by excitement or fear, which, of course, are to be differentiated from the effects produced by the intravenous injection of solutions of either drug.

In this connection mention may also be made of the experiments of Joseph,[88] who found that arsphenamin is practically without irritation when applied directly to nerve and muscle tissue of the frog. Also of the study by Perutz and Rosenmann,[89] who found that ordinary doses of neoarsphenamin had no toxic effect on the action of the extirpated gut of warm blooded animals, although some effect was produced upon the gut of cold-blooded animals.

Influence of Arsphenamin and Neoarsphenamin upon Blood Chemistry and Kidney Elimination.—Investigations bearing upon the changes that may be produced by arsphenamin and neoarsphenamin on the normal

chemical constituents of the blood have been particularly interesting in relation to the toxicological effects that may be produced by these drugs in the treatment of syphilis.

Marischler and Schneider[90] investigated three cases of syphilis; one primary, one secondary and one tertiary after the intramuscular injection of acid solutions of arsphenamin. An increased excretion of calcium oxid and phosphorus in the urine and feces was found in all three cases, and in one case there was an increase in the phosphorus to nitrogen ratio, with a corresponding decrease in the nitrogen output. The authors concluded that arsphenamin acts like an acid, combining with the alkali of the body, especially with the calcium of the bone cells.

Rappleye,[91] studied the effects of intravenous injections of arsphenamin in alkaline solution on blood urea and the phenolsulphonephthalein elimination in paretic patients, concluding that arsphenamin has no injurious effects upon the kidneys when their function is good at the outset. Elliott and Todd[92] made similar observations as likewise Anderson,[93] Weiss and Corson;[94] the latter made particularly careful determinations of non protein nitrogen, sugar, uric acid, carbon dioxid combining power of the plasma and elimination of phenolsulphonephthalein. Finally Raiziss and Brown have determined the effects of single large and multiple small doses of arsphenamin (disodium salt) and neoarsphenamin upon the total non protein nitrogen, urea nitrogen and creatinin of the blood of rabbits before and at varying intervals after the intravenous injection of the drugs, with the general result that while the single massive doses of both compounds produced marked nephritis with an increase of non protein nitrogen, urea and creatinin, twelve small doses in amounts corresponding to the amounts ordinarily administered human subjects in the treatment of syphilis, had no appreciable effects. McFarland[96] in a study of the cholesterol of the blood in syphilitic patients found that, in general, the amounts tend to be medium and low rather than high, the results being similar to those expected in a similar series of non-syphilitic individuals, except in some cases of neurosyphilis when the cholesterol content appeared to be increased. The administration of arsphenamin, however, had no effect upon cholesterol content of the blood. Bailey and MacKay[97] however, found hypercholestermia an early and marked sign of toxic jaundice sometimes associated with an increase of uric acid of the blood; studies were also included of bile pigments and salts in the urine and plasma.

It may be stated, therefore, that the administration of arsphenamin and neoarsphenamin in ordinary therapeutic doses at the usual intervals of injection, have no appreciable effects upon the blood constituents or kidney elimination, if the kidneys are functioning normally when treatment is begun. It is, of course, well known that arsphenamin and neoarsphenamin may produce nephritis with changes in elimination but under ordinary circumstances these accidents do not occur and the drugs produce none or but minor and unimportant changes in the blood chemistry and in kidney elimination in the majority of cases of syphilis undergoing treatment with intravenous injections of arsphenamin and neoarsphenamin. In other words these drugs are not ordinarily injurious to the kidneys although other organic and some inorganic arsenical compounds are more renotropic than these drugs.

In this connection I may also state that while Rowe[98] and Tokuda[99] have observed interesting changes in the refractometric indices of the serum in syphilis, the latter has observed that neither arsphenamin nor neoarsphenamin produced any marked or constant influence. Of further interest in this connection was a similar refractometric study by Zimmer[100] to determine

the nature of the increase in weight of syphilitic patients under arsphenamin treatment. Zimmer found that the increase may be true and due to an increase of substance rather than a pseudo-increase from water retention, although sudden increases on weight may be due to retention of water in the blood and tissues as shown by changes in the protein content of the serum. In general, however, the author concludes that arsenic may change the balance between blood and tissues with an increased capacity for mobilization of the tissue fluids in acute osmotic disturbances.

Influence of Arsphenamin and Neoarsphenamin upon the Corpuscular Elements of the Blood.—Arsphenamin and neoarsphenamin are probably erythrogenic to a slight degree, that is, capable of stimulating the bone marrow with an increased production of erythrocytes. In cases of syphilitic anemia, I have frequently observed a steady rise in the number of erythrocytes to normal proportions along with an increase of hemoglobin during treatment with intravenous injections of neoarsphenamin at weekly or biweekly intervals. Likewise in the primary anemias of non-syphilitic origin, the administration of neoarsphenamin is sometimes followed by slight but definite increase of erythrocytes, although the evidences of bone-marrow activity are not as definite as has been observed with germanium. Doubtless the improvement in the anemia of syphilis is due in part to the destruction of the parasites and the removal thereby of their hemotoxins. In chronic syphilis with normal blood pictures, the administration of arsphenamin, neoarsphenamin and substitute drugs are without appreciable influence upon the erythrocytes and hemoglobin; in other words, the effects of arsphenamin and neoarsphenamin in ordinary and usual doses upon erythrocytes and hemoglobin of syphilitic individuals are apparent only when an anemia exists.

Ordinarily an intravenous injection of arsphenamin or neoarsphenamin has no constant influence upon the total or differential leukocyte counts when reactions do not occur. An individual with about 7000 cells per cubic millimeter may show an increase to approximately 9000 when counted within an hour or two after an injection, but at the end of twenty-four hours the count is usually at the pre-injection level. Intramuscular injections of sulpharsphenamin have usually produced a mild leukocytosis (9000 to 12000 cells per c. mm.) at the end of twenty-four hours, along with a slight increase of the polymorphonuclear neutrophilic leukocytes, but these effects are probably due to local irritation of the drug. However, when reactions follow the administration of arsphenamin or its substitutes, and especially in dermatitis, more marked changes in the blood may occur and especially a decrease of the polymorphonuclear neutrophils along with an increase of the large mononuclears and eosinophils. I have observed these changes in the dermatitis so frequently developing after the intravenous or intramuscular injections of sulpharsphenamin and will refer to them again with more detail in Chapter LI, devoted to a discussion of the toxicological effects of arsphenamin and its substitutes, as likewise the aplastic anemias characterized by leukopenia, eosinophilia, lymphocytosis, etc., due to toxic destruction of the bone marrow sometimes produced by arsphenamin.

Effects of Arsphenamin and Neoarsphenamin upon the Coagulability of the Blood.—Ordinary therapeutic doses of arsphenamin do not appear to alter the coagulation time of the blood of human subjects but large doses may produce profound changes in experimental animals. Flandin and Tzanck[101] for example, have called attention to the incoagulability of the blood of animals receiving large doses of arsphenamin, and from certain experiments came to the conclusion that the lack of coagulation was due to the action of the arsphenamin on thrombin or its precursor.

In a thorough study of this subject Oliver and Douglas[102] came to the conclusion that arsphenamin may produce incoagulability of the blood both in the test tube and rabbit by acting chiefly on the fibrinogen (globulins), rendering the blood incoagulable to heat or thrombin. No action on thrombin could be demonstrated as claimed by Flandin and Tzanck. While the effects observed on the properties of the globulins was similar in nature to that caused by alkali, yet the action of the alkali alone will not explain the results obtained according to Oliver and Douglas, with solutions of disodium arsphenamin. Oliver and his colleagues also found that arsphenamin administered in gelatin solutions did not have these effects, due to the protection afforded by the colloid.

In repeating some of Olivers' experiments I have observed similar effects. The intravenous injection of rabbits with large doses of disodium arsphenamin in saline (0.100 gm. per kilo) invariably rendered the blood incoagulable, whereas similar doses administered in 0.5 to 2 per cent. solutions of gelatin had no such effects.

Neoarsphenamin may likewise reduce the coagulation time of the blood although this subject has not been thoroughly studied. Some years ago Dr. Lake of the Hygienic Laboratory drew my attention to the fact that rats injected with large amounts (0.200 gm. or more per kilo) in the conduct of toxicity tests, not infrequently continued to bleed for an hour or more after injection. This observation has been amply confirmed and the changes in coagulation time are so marked that it is sometimes necessary to ligate the vein after the injection of these animals. With human subjects receiving 0.9 gm. intravenously I have sometimes observed that the coagulation time measured with the Bogg's coagulometer was delayed one to several minutes beyond the pre-injection time, although usually within four hours the delay in coagulation had disappeared.

Effect of Arsphenamin and Neoarsphenamin upon the Epinephrin Content of the Adrenal Glands.—As discussed with more detail in Chapter LI, it is commonly believed that the administration of arsphenamin and neoarsphenamin may bring about changes in the epinephrin content of the blood, since the administration of this substance may sometimes relieve the acute symptoms of the immediate arsphenamin reaction or "nitritoid crisis."

As shown by Brown and Pearce[103] and confirmed by Lucke and myself,[104] the administration of very large amounts of arsphenamin and neoarsphenamin to rabbits and rats may produce histological changes in the adrenal glands, and Hirano,[105] after a study of the epinephrin content of rabbits' adrenals by the colorimetric method of Comessatii, after the intravenous injection of arsphenamin and neoarsphenamin, concluded that large doses of these arsenicals produced a remarkable decrease of chromaffin substance and of residual epinephrin, and that in therapeutic doses arsphenamin caused partial exhaustion, while neoarsphenamin did not influence the epinephrin contents.

In a study of this subject, employing rabbits, by Lucke, McCouch and myself,[106] it was found that doses corresponding to those employed in the treatment of syphilis had no appreciable effects. The adrenal glands were extracted according to the method of Folin, Dennis and Cannon[107] and asayed by the biological method of Elliott.[108] Rabbits injected with doses of 0.02 to 0.1 gm. per kilogram corresponding to 1.2 to 6.0 gm. per 60 kilograms, showed a considerable range of values, but on the average the amounts were almost identical with the average of the epinephrin values of control rabbits.

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CHAPTER XLIV

GENERAL CONSIDERATIONS WITH ETIOLOGICAL AND CLINICAL CLASSIFICATIONS OF THE TOXIC REACTIONS PRODUCED BY ARSPHENAMIN, NEOARSPHENAMIN AND OTHER ORGANIC ARSENICAL COMPOUNDS

Even before organic compounds of arsenic were employed for medicinal purposes, the toxicological effects of the inorganic salts commanded a great deal of attention for hundreds of years and especially in relation to chronic poisoning. During the last twenty years, it has been shown that accidental arsenical poisoning is not infrequently due to the widespread presence of the element in beverages, foods, cosmetics and other substances in common use, but, since the introduction of organic arsenical compounds for the treatment of trypanosomiasis, syphilis, and other diseases, interest in the toxicological or untoward effects has been greatly increased and especially since the discovery of arsphenamin and neoarsphenamin. I do not know of any other group of compounds which have been subjected to as much laboratory and clinical study in relation to toxicological effects as these two arsenical substances and since 1910, an enormous literature has accumulated upon the subject.

Importance of the Subject of Toxic Reactions in Relation to the Science of Chemotherapy.—Clinical and laboratory experience with atoxyl and arsenophenylglycin had lead Ehrlich and his associates to expect that untoward effects may be observed in at least some cases of syphilis treated with arsphenamin and especially evidences of neurotropism, but in the early days of salvarsan therapy no one surmised that the subject would assume the importance that subsequent events have developed. Indeed, laboratory and clinical investigations upon the toxicity of arsphenamin and its substitutes have very materially modified early conceptions of the mechanism of toxicity

of drugs in general and have shown that it is not merely a matter of what may happen when a drug has been brought into contact with the fixed cells of various organs, but that other and important reactions may occur in the blood and body fluids before this union occurs and especially if the medicinal compound is administered by intravenous injection.

Few chapters in the history of chemotherapy are as interesting and important as that concerning the toxicology of arsphenamin, neoarsphenamin and their substitutes, not only from the practical standpoint in relation to the treatment of syphilis and other diseases, but also in relation to the science of chemotherapy in general, of which mention may be made of the influence of reactions between the blood and solutions of the drug, the histopathological changes in important organs that may be produced by the drug, the influence of errors in administration, the modifying influence of congenital or acquired hypersensitiveness to the drug, the influence of pathological changes in important organs that may be produced by syphilis and other infections, etc., as examples of the numerous factors to be studied and taken into consideration in relation to the possible toxicological effects of a chemical substance employed in the treatment of disease. In other words, our present knowledge of the manifestations and mechanism of the toxicological effects that may be produced by the organic arsenical medicinal compounds serves as an example of the difficulties to be confronted in chemotherapeutic research and indicate especially that the minimal lethal and maximum tolerated doses of a compound for the *healthy* lower animals are only a part—a small, although a valuable and essential part—of the data required for evaluating a remedy from the standpoint of its possible untoward and harmful effects for the *sick* human being.

Organic Arsenical Compounds of Special Interest from the Standpoint of Toxic Reactions.—Of the very large number of trivalent and pentavalent organic arsenical compounds which have been described since arseno compounds were first prepared by Michaelis in 1881 and recently compilated by Raiziss and Gavron,[1] only those which have proven sufficiently low in toxicity and high in parasiticidal activity to gain a place in the treatment of diseases of human beings or the lower animals require consideration in this treatise. First among these in practical importance are the trivalent compounds arsphenamin, neoarsphenamin, sodium arsphenamin and sulpharsphenamin; among the coördination compounds, only silver arsphenamin has proven worthy of inclusion; among the pentavalent compounds, only atoxyl and tryparsamid require discussion as the others possess no material superiority over these in medicinal value.

In a general manner, the toxic manifestations of these different compounds have certain features in common since the arsenic of all exerts a similar pharmacodynamic effect. Of course, the chemistry of the different compounds exerts important special effects, not only as influencing the effects of arsenic, but also the kinds and amounts of impuritites which may be present by reason of technical factors in their preparation or administration, because of varying primary physicochemical effects upon the blood when administered intravenously, varying selective affinities for certain tissues, etc. For example, solutions of arsphenamin are acid and when injected into the blood in this state without neutralization may produce profound and dangerous physical changes and toxic reactions; some of the pentavalent arsenicals like atoxyl have special affinity for nerve tissue (neurotropism), etc.

Etiological Classification of the Toxicological Reactions Following the Administration of Arsphenamin, Neoarsphenamin and Other Organic Arsenical Compounds.—In a broad and general manner, it may be stated

that the causes, lesions and symptoms of the toxicological effects of arsphenamin and other organic arsenical compounds are numerous and exceedingly complex; I know of no other class of compounds quite as complicated from these standpoints and concerning which so many and diverse factors require consideration.

In the first place, arsphenamin, neoarsphenamin and most organic arsenicals are exceedingly complex chemical compounds, are not crystallizable and therefore difficult or impossible of preparation on a large scale in an absolutely pure state with uniform biological qualities. Even when manufactured with the utmost skill and care and apparently in a perfectly uniform manner arsphenamin and especially neoarsphenamin, prepared in different laboratories as well as different batches in the same laboratory, are apt to vary in toxicity and therapeutic activity. From the standpoint of physical appearance the products may be quite excellent and acceptable and yet produce annoying or even serious untoward effects when administered to human beings. Some of these effects may be unavoidable, even when the technic of administration is perfect; for example, arsphenamin itself is hemotecic even in isotonic solutions and the effects of intravascular hemolysis may be apparent in some cases by producing the non specific protein type of reaction. Furthermore, a perfectly good compound may produce Jarisch-Herxheimer reactions which are not to be ascribed to a toxic medicament although the reaction may be erroneously regarded as a toxicological effect.

Not infrequently and indeed usually, rigid tests for toxicity in rats and rabbits may fail to detect the presence of the toxic substances, because of a difference in reaction between these healthy lower animals and sick human beings and chemical analyses of the products are notoriously deficient for detecting and identifying impurities that may be held responsible. Nevertheless, there can be no doubt that toxic substances may be present in the medicament responsible for some of the untoward effects even though these cannot be identified with certainty by the biological and chemical tests in present use.

However, it should be stated that the manufacture of arsphenamin and neoarsphenamin has been brought to such a state of perfection that products fulfilling the toxicity standards of the Hygienic Laboratory are not usually to be held responsible for untoward toxic effects, although it is possible that impurities may gain access during manufacture which escape detection in animal tests and chemical assays. In my opinion these impurities are chiefly arsenical substances, since the toxicological reactions present the lesions and symptoms of arsenical poisoning. Furthermore, individual ampules of arsphenamin and especially of neoarsphenamin may acquire toxicity after leaving the manufacturer by reason of oxidation or other changes of deterioration which always renders advisable the routine use of fresh neoarsphenamin; here again I believe that these toxic substances are arsenical compounds rather than the methyl alcohol, sulphur compounds, etc., that are usually present in varying amounts.

As would be expected, susceptibility to the effects of these toxic arsenical substances as well as to the normal oxidation or reduction products of the organic arsenical compounds formed in the body after administration, varies among different individuals so that many factors relating to the patient require consideration. Fear and nervousness, the existence of general debility or some other disease and especially of the gastrointestinal tract and liver, defective elimination of arsenic as a result of disease of the liver, kidneys or both, congenital or natural allergic hypersensitiveness, focal

infections, etc., are some of the factors usually requiring consideration in arriving at conclusions regarding the etiology of a toxicological reaction in any given case or group of cases.

Finally, the untoward toxic effects of arsphenamin, neoarsphenamin and other organic compounds of arsenic may be due to factors concerned in the preparation and administration of the drugs. This is especially true of arsphenamin, because solutions of the drug require alkalinization or, at least, neutralization before intravenous injection and because faults in this part of the technic as well as over alkalinization and faults in the degree of dilution and rapidity of injection may bring about acute reactions in the blood. It was quite the custom of Ehrlich to blame the toxic reactions of arsphenamin upon faults in its administration, and present-day manufacturers are likewise liable to think first of some error in administration on the part of the physician when reactions follow the administration of their products; but such are not always the case.

It is apparent, therefore, that the toxic or untoward reactions following the administration of arsphenamin and its substitutes may be due to three primary factors, namely, (*a*) to the drug itself or some impurity it may contain; (*b*) to pathological states in the individual and variations from the normal in susceptibility to the toxic effects of the drugs and impurities and (*c*) to errors in administration. Of these three general causes, I believe that the most frequent and important are those due to factors involving the individual patient followed second in importance and frequency, by errors in the technic of administration and lastly, the toxicity of the arsenical derivatives produced in the body from the drugs themselves or to toxic arsenical compounds or other impurities gaining access to the drug before or during its administration.

The following summary of these various causes of the toxicological reactions that may follow the administration and especially the intravenous injection of the organic arsenical compounds shows how complicated the subject may be and many of these factors are worthy of more detailed discussion before the lesions, symptoms and treatment of the untoward reactions themselves are considered.

CLASSIFICATION OF CAUSES OF TOXICOLOGICAL REACTIONS FOLLOWING THE ADMINISTRATION OF ARSPHENAMIN AND NEOARSPHENAMIN

1. Due Primarily to the Drug
   1. Due to toxic physical properties of solutions.
   2. Production of agglutination of erythrocytes and precipitation of plasma constituents.
   3. Production of hemolysis.
   4. Toxic chemical effects of arsenical oxidation or reduction compounds.
   5. Toxic chemical effects of arsenic.
   6. Toxic chemical effects of non-arsenical impurities.

2. Due Primarily to the Patient
   1. Fear and nervousness.
   2. Endocrine disturbances; vagatonia.
   3. Focal infections.
   4. Pathological changes due to syphilis.
   5. Production of Jarisch-Herxheimer reactions.
   6. Production of syphilitic recurrences.
   7. Pathological changes due to non-syphilitic diseases.
   8. Pathological changes due to mercury.
   9. Natural or acquired hypersensitiveness to arsenical compounds.

3. Due Primarily to Method of Administration	1. Failure to neutralize solutions of arsphenamin. 2. Over-alkalinization of solutions of arsphenamin. 3. The use of defective water or saline for preparing solutions. 4. Excessive dilution. 5. The intravenous injection of too concentrated solutions. 6. Too rapid intravenous injection. 7. Oxidation and other physical and chemical changes in solutions. 8. The use of new rubber tubing. 9. Emboli of undissolved drug, cotton, air, etc. 10. Accidental injection of perivascular tissues.

Clinical Classification of the Toxic Reactions Following the Administration of Arsphenamin, Neoarsphenamin and Other Organic Arsenical Compounds.—With so many possible causes for toxic reactions following the administration of arsphenamin and its substitutes, it is no wonder that the lesions and symptoms may be many and very diverse in character and severity. Indeed, there seems to be an almost endless variety of symptoms that may be observed in different cases and the best one can do is to discuss certain types of reactions realizing at the outset that individual patients may show many deviations due to intrinsic factors concerning the particular drug used, its preparation and administration, not to mention the tremendous influence that may be exerted by the general condition of the patient at the time of administration.

In 1916, Schamberg, Raiziss and I[2] proposed a classification of the symptoms according to the time of onset after injection into immediate, early and late groups; since then various additional classifications have been proposed by Sicard and Roger,[3] Martin,[4] Fraser[5] and others, but I propose the following as being quite serviceable:

CLINICAL CLASSIFICATION OF TOXIC REACTIONS FOLLOWING INTRAVENOUS INJECTIONS OF ARSPHENAMIN AND ITS SUBSTITUTES

1. Immediate Reactions (During or Immediately after Injection)	1. The acute physico-hemoclastic reaction. 2. The acute vasoparetic reaction or "nitritoid crisis" (*a*) Mild. (*b*) Severe.
2. Early Reactions (Following within 24 Hours after Injection)	1. The gastro-intestinal reaction. 2. The protein and colloidal shock reaction. 3. The accidents of injection as phlebitis, thrombosis, perivascular infiltrations, etc.
3. Late Reactions (Occurring One Day to Several Weeks after Injection)	1. Hemorrhagic encephalitis. 2. Dermatitis and allied reactions. 3. Neuritis, neuro-recurrences and neural Herxheimer reactions. 4. Jaundice.

The etiology, lesions, symptoms and treatment of these various syndromes are discussed in Chapter LI, as likewise the few mild untoward effects that may commonly accompany any intravenous injection due to nervousness and fear on the part of the patient and not to toxic effects of the drug administered.

Frequency of Reactions Following the Administration of Arsphenamin and Its Substitutes.—It is not possible to express in accurate terms the frequency of all reactions following the administration of arsphenamin, neoarsphenamin and other organic arsenical compounds employed in the treatment

of syphilis. So many factors may be concerned, referable not only to the toxicity of the different drugs themselves but also to the skill and experience of the physician in preparing and injecting the solutions, that the incidence of reactions is subject to wide and varying fluctuations. However, I believe that it may be stated that the incidence is much higher for arsphenamin than for neoarsphenamin and with both drugs is generally lower at the present time than five to ten years ago, due to an increasing knowledge of the mechanism of the reactions and means for their prevention.

Influence of the Kind of Organic Arsenical Compound Administered upon the Incidence of Toxic Reactions.—At the present time arsphenamin, neoarsphenamin and sulpharsphenamin are being mostly employed in the treatment of syphilis. In regard to toxic reactions it may be stated that in the majority of cases they do not occur at all when the compounds are administered in the usual doses and by technically acceptable methods. This is especially true of the better brands of neoarsphenamin; arsphenamin produces more reactions, not only on account of its higher arsenic content but also because of toxic physical factors involving the solutions, the greater chances of producing agglutination and hemolysis of erythrocytes, the use of larger amounts of water, errors in the neutralization and alkalinization of the solutions, etc.

With present-day arsphenamin administered in dose of 0.4 to 0.6 gm. by intravenous injection to adults according to the technic described in Chapter LIX, the general incidence of reactions irrespective of sex, number of previous injections and stage of the disease and including such mild reactions as headache, nausea and diarrhea, is about 10 to 20 per cent. Neoarsphenamin in dose of 0.6 to 0.9 gm. by intravenous injection to adults according to the technic described in Chapter LIX, produces not more than 2 to 10 per cent. of reactions. In other words, the incidence of untoward reactions following the administration of arsphenamin has been in my experience at least 4 to 10 times higher than produced by neoarsphenamin; Ormsby has[6] reported an incidence of about 20 per cent. reactions following the administration of arsphenamin and about 8 per cent. after neoarsphenamin. Merritt[7] states that a reaction of some sort, although often slight, is to be expected in from one-third to one-fifth of all injections of neoarsphenamin, the incidence being still higher for arsphenamin. In dispensary practice the incidence is always likely to be higher than in private practice; for example, reactions of some sort (usually mild) occurred in 100 or 43 per cent. of 233 patients treated with arsphenamin in the clinic of Dr. Schamberg and in 486 or 39 per cent. of 1217 patients treated with neoarsphenamin. These figures are based almost entirely upon the reports of patients upon the occurrence of fever, chills, headache, nausea, vomiting or diarrhea after injection; not infrequently the only symptom reported was headache which obviously may have been due to some other factor. In other words these figures are certainly too high for the true incidence of reactions since symptoms of the kind reported may not have been due to the drugs at all. As reported, however, by these adult dispensary patients, the incidence of the various symptoms were as follows, there being but slight differences in the incidences of reactions in both sexes:

	Headache (Per Cent.)	Nausea (Per Cent.)	Vomiting (Per Cent.)	Diarrhea (Per Cent.)	Fever (Per Cent.)	Chilliness (Per Cent.)
Arsphenamin.....	60–70	46–54	14–26	36–50	30–40	38–40
Neoarsphenamin...	64	50–68	20–25	37–39	27–31	24–28

Sulpharsphenamin administered by subcutaneous, intramuscular and intravenous injection has produced in my practice a higher incidence of dermatitis than either arsphenamin or neoarsphenamin, although I have not administered this compound to a sufficient number of cases to express even an approximate estimate of the incidence of this and other toxic reactions.

As stated in Chapter LI, the incidence of the severer reactions as exfoliative dermatitis, jaundice and the so-called hemorrhagic encephalitis is much lower. Sulpharsphenamin, like arsenophenylglycin, is somewhat more likely to produce dermatitis than arsphenamin and neoarsphenamin, although the products marketed during the past two years have been in my experience much less toxic in this regard than those formerly prepared. The incidence of jaundice has fluctuated greatly in different years due, presumably, according to Stokes and others, to the fact that jaundice may be caused by co-incident infections rather than by the drug. Fatal cases of hemorrhagic encephalitis have been quite rare in America; most cases on record have occurred in Europe.

Furthermore, the incidence of reactions varies considerably according to the brand of arsphenamin and neoarsphenamin administered and, indeed, may vary with different lots of the same compound prepared by the same laboratory. There is no uniformity at all in the experience of physicians with these compounds; some observe a high incidence of toxic reactions with one brand which the physician next door may be using in approximately the same dosage with very few reactions. It is well nigh a hopeless task even to try to reconcile or offer an explanation of the remarkable variations in toxic reactions due to these compounds in the experience of different physicians, because of the large number of factors requiring consideration, but dosage and technic of administration are of more importance than the brand of arsenical employed.

Influence of Dosage upon the Incidence of Toxic Reactions.—Needless to state the incidence of toxic reactions is greatly influenced by dosage. For example, the administration of 0.1 to 0.3 gm. of arsphenamin or 0.2 to 0.4 gm. of neoarsphenamin are only occasionally followed by reactions and not infrequently the absence or low incidence of toxic reactions in the practice of some physicians is found upon inquiry to be due to the fact that they are injecting smaller doses than generally employed and under these circumstances, together with the use of good methods for preparing and administering solutions of the drugs, it is not difficult to understand how or why untoward effects are seldom observed.

Undoubtedly the subject of dosage has not received the attention that its importance demands in relation to the treatment of syphilis and more especially in relation to the toxic reactions. Too many physicians administer arsphenamin and neoarsphenamin by rule instead of gauging dosage and intervals of injection according to the condition of each individual patient. In the treatment of acute early syphilis of otherwise healthy vigorous subjects, the usual maximum doses of 0.6 gm. arsphenamin and 0.9 gm. neoarsphenamin are indicated but in the treatment of chronic syphilis the condition of the kidneys, liver, cardio-vascular system, etc., must be taken into account. It appears that most of the fatalities ascribed to arsphenamin and its substitutes have occurred among individuals with advanced pathological changes in the internal organs to whom the drugs should not have been given at all or in but small and cautious doses. Indeed, the salvarsan committee of the Allgemeiner aerztlicker Verein of Munich went so far in 1920 as to recommend that manufacturers should not be allowed to place more than 0.6 gm. of neoarsphenamin in a tube as representing the maximum

single dose to be given at one time, since in their analysis of fatalities it was found that the incidence rose sharply with larger doses. However, this point should not be over-emphasized; as shown by Wechselmann, a dose of 0.1 or 0.2 gm. of arsphenamin may be dangerous for some individuals and especially in the presence of nephritis whereas 0.6 gm. is safe for the majority. In other words, the question of dosage bears a very important relation to the question of individual susceptibility and tolerance which is discussed with more detail in Chapter XLVII.

Influence of Dosage upon the Toxicity of Arsphenamin and Neoarsphenamin for Rats.—As would be expected, the resistance of rats to the effects of arsphenamin and neoarsphenamin likewise varies greatly according to the amounts administered per kilogram of body weight. Individual variations in resistance among these animals have been noted similar to individual variations among human beings, but by keeping the animals on a uniform diet, avoiding the injection of pregnant females and selecting animals of nearly uniform age and weight it is possible to bring out rather sharply the influence of dosage upon the incidence of fatal toxic reactions. An analysis of my records covering toxicity tests of 181 different lots of arsphenamin and 278 lots of neoarsphenamin conducted several years ago have shown the following results; the great majority of animals were of the regulation weight (100 to 150 grams) with a few about 90 grams and a few as high as 170 grams. The injections were intravenous and the general technic exactly as described in Chapter II.

	Arsphenamin	Neoarsphenamin
Average highest dose per kilo killing at once	0.150	0.367
Average highest dose surviving for 1 day	0.133	0.343
Average highest dose surviving for 2 days	0.129	0.340
Average highest dose surviving for 5 days	0.128	0.318
Average highest dose surviving for 7 days	0.128	0.317
Average highest dose surviving for 10 days	0.128	0.316
Average highest dose surviving for 14 days	0.127	0.315

It will be noted that with arsphenamin the highest percentage of deaths occurs in the first forty eight hours after injection and after this time the percentage of survivals remains quite constant; with neoarsphenamin however, the highest percentage of deaths is not reached until 5 to 10 days after injection. These results are in confirmation of those observed by Lake and Roth in the Hygienic Laboratory and support the statements of this laboratory that in toxicity tests of arsphenamin the results may be evaluated at the end of 48 hours whereas in tests with neoarsphenamin a period of at least 5 to 7 days must elapse after injection.

Influence of Sex upon the Incidence of Toxic Reactions.—Women are more likely to develop reactions than men. In my experience the mild reactions of nausea, headache, fever, diarrhea, etc., following injections of arsphenamin are as 5:4 as compared to men and similar reactions after injections of neoarsphenamin are almost 2:1 as compared with the incidence among men. These differences are probably due to the fact that, although women as a group exhibit less fear and nervousness concerning the operation of injection, yet their nervous systems are more susceptible to shocks and they are likewise more subject to endocrine disturbances, constipation and other predisposing causes. The incidence of jaundice is somewhat higher among

women than men due in part at least to the commonly observed fact that the incidence of cholelithiasis and various disorders of the liver and bile ducts is higher among them than among men.

The incidence of fatalities following injections of arsphenamin and its substitutes has been, however, higher among men, but this is probably due to the fact that more men than women undergo treatment for syphilis. However, while women tend to develop the minor toxic reactions more frequently than men as stated above, it may be that they are actually somewhat more resistant to the severer toxic reactions. In this connection the results of my toxicity tests with male and non-pregnant female rats are not without interest:

In determining the toxicity of 71 different lots of arsphenamin, 185 male and 175 non-pregnant female rats of regulation weight were employed. The dose injected intravenously was 0.100 per kilo as described in Chapter II. Among the males there was 76 per cent. survivals for two days and among the females, 85 per cent. survivals.

In toxicity tests of 66 different lots of neoarsphenamin, 242 male and 173 non-pregnant female rats were injected intravenously with 0.200 gm. per kilo of weight; 79 per cent. of the males survived for 7 days while 84 per cent. of the females survived.

These results have shown, therefore, that non-pregnant female rats are somewhat more resistant to the toxic effects of arsphenamin and neoarsphenamin than male rats of the same weight, kept under the same conditions and injected in exactly the same manner at the same time; I believe that the same conditions pertain to human beings in that in a broad and general manner women are more resistant to the severer toxic reactions of arsenic than men.

Influence of the Number of Injections upon the Incidence of Reactions.—In my experience the incidence of reactions is generally higher after five or more injections of arsphenamin or neoarsphenamin have been given than after the first injection. Ormsby however, states that in his experience the incidence following the administration of neoarsphenamin, has been higher after the first injection and Leredde in 1914 pointed out that most of the reported fatalities ascribed to salvarsan occurred after the first injection because Jarisch-Herxheimer reactions are more likely to occur after the first than after subsequent injections. In my experience however, there is likewise a greater tendency for reactions during the second or subsequent "courses" of injections of arsphenamin and neoarsphenamin than during the first course.

It is highly probable that these effects are due to the accumulation of arsenic in the liver and other organs or to the production of tissue injuries resulting in interference with the metabolism and elimination of the compounds as treatment progresses. In some cases it is also apparently due to the acquisition of allergic sensitiveness to the drugs. In those rare instances of natural allergy to arsenic the first injection may of course produce more reaction than subsequent injections if a continuation of treatment with the same compound is attempted. One may also consider the possibility of more reaction from the first than from subsequent administrations due to the sudden destruction of large numbers of spirochetes with the production of toxic disintegration substances, but I believe this factor is far over-rated in importance.

Cumulative toxic effects due to the retention of arsenic as treatments are continued along with the possibility of these lowering general resistance to bacterial infections by histological changes in the bone marrow and other organs as well as by tissue changes in the liver, kidneys, etc., are more likely to be responsible for toxic reactions and especially the severer ones as dermatitis and hepatitis.

Influence of Age and Weight upon the Incidence of Toxic Reactions.—It is now well known that children bear the administration of arsphenamin and its substitutes in doses according to body weight much better than adults;

the same is true of the inorganic compounds. Even in 1786 Fowler[8] stated in his original monograph on the use of his solution, that "I observed that children were less apt to suffer from the medicine than adults."

This increased tolerance of children to arsenic is possibly due to several factors. In the first place the studies of Benedict[9] and of Benedict and Talbot[10] have shown that the metabolic rate of young animals is greater than that of old animals since the active mass of protoplasm which determines fundamental metabolism decreases with advancing age. Moreover, the blood volume decreases with advancing age and these two factors apparently reduce the facility of older animals for getting rid of toxic substances. In addition very young children are usually treated with intramuscular injections of the arsenicals which are less apt to produce toxic reactions than intravenous injections. Furthermore, children are usually in better general physical condition and free of predisposing states to toxic reactions as vagatonia, constipation, focal infections, hepatic and renal disorders, etc.

Influence of Age and Weight upon the Resistance of Rats to Arsenical Compounds.—Exactly similar conditions have been observed in rats treated with intravenous injections of various arsenical compounds, that is, young rats are more resistant than older animals. A few years ago it was shown by Roth[11] in experiments with white rats injected intravenously with arsphenamin and neoarsphenamin, that young rats are much more resistant than old and heavier animals. For example, of rats below regulation weight (50 to 100 grams) injected intravenously with 0.140 gm. of disodium arsphenamin per kilogram of weight, 53 per cent. survived for forty-eight hours; of rats weighing from 100 to 150 grams (regulation weights), 28 per cent. survived, whereas in one series of rats much above regulation weight (180 to 275 grams) none survived. Similar results were observed with neoarsphenamin since 82 per cent. of young rats weighing from 42 to 70 grams survived for seven days or longer the intravenous injection of 0.240 gm. per kilogram of body weight whereas only 67 per cent. of older rats of regulation weight (101 to 135 grams) survived.

An analysis of my records of toxicity tests with arsphenamin and neoarsphenamin, conducted as described in Chapter II, have shown similar results. For example, in testing 100 different lots of arsphenamin 526 rats were injected intravenously with 0.100 gm. per kilogram of weight. Of the animals weighing from 90 to 125 grams, the survivals for two days was 72 per cent.; of the animals weighing from 130 to 150 grams the survivals was 71 per cent., while among slightly heavier animals weighing from 160 to 180 grams the survivals was 58 per cent. In tests of 105 different lots of neoarsphenamin 592 rats were injected intravenously with 0.200 gm. per kilogram of weight. Of the animals weighing from 90 to 125 grams, the percentage of survivals for 7 days was 82 per cent.; of the animals weighing from 130 to 190 grams, the survivals was 78 per cent.

Hammett and Nowrey[12] have observed a still more striking influence of the age of rats upon resistance to injections of arsenic trioxid. For example, among rats 60 to 90 days old and weighing from 100 to 137 grams, the fatal dose was 0.0011 gm. or over per kilogram of weight; among rats 120 to 150 days of age and weighing from 124 to 175 grams, the fatal dose was 0.009 gm. per kilo; while among rats 210 to 240 days of age and weighing from 161 to 223 grams, the fatal dose was 0.008 gm. per kilo of weight showing a progressive decrease in resistance with advancing age. Hammett and Nowbrey ascribe the results in large part to differences in metabolic rates, pointing out that the mass of active protoplasm concerned in getting rid of noxious agents decreases with advancing age and calling attention to the observations of Donaldson[13] and Jackson[14] that the viscera decrease in their percentage relation to body weight with advancing age.

Incidence of Fatalities Ascribed to the Administration of Arsphenamin and Neoarsphenamin.—Since the introduction of salvarsan for the treatment of syphilis in 1910 by Ehrlich, millions of doses have been administered without serious mishaps. When one pauses to consider the potential dangers connected with its administration by the thousands of physicians the world over and especially the accidental injection of acid solutions and likewise the fact that thousands of individuals with advanced heart and kidney disease as well as numerous cases in extreme states of debility due to syphilis or other pathological states have been injected almost indiscriminately, the wonder is not that fatalities have occurred but that the incidence is so low in comparison to the number of injections given.

In 1913 Leredde[15] states that the deaths from salvarsan were approximately as follows:

1910	50,000 injections—16 deaths	(1:3000)
1911	800,000 injections—92 deaths	(1:8700)
1912	1,280,000 injections—66 deaths	(1:19000)
1913	2,000,000 injections—37 deaths	(1:54000)

These figures were interpreted as indicating that a large proportion of deaths were due to faulty technic in administration and disregard of contraindications, rather than to toxicity of the drug, since the incidence decreased as experience with it was gained. Arranged according to the number of injections received by the patient, the table shows that of 211 cases:

104 occurred after the 1st injection
55 occurred after the 2nd injection
13 occurred after the 3rd injection
8 occurred after the 4th injection
1 occurred after the 5th injection
1 occurred after the 6th injection
1 occurred after the 7th injection

The decrease of fatalities after the first injection, without a comparable decrease after the second injection, was ascribed by Leredde to the fact that Herxheimer reactions were responsible for many fatalities and that it occurs most frequently at that time.

The total of reported fatalities following injections of salvarsan up to 1914 was approximately 274 cases; in Schmitt's[16] analysis of these, it was found that in a large proportion there was no connection between the drug and the fatalities, while in others the dosage was excessive. Benario[17] goes further and states that of these 274 cases only from 4 to 6 could be ascribed to salvarsan given by a proper technic. In the 41 cases in which the drug had been given to non-syphilitics, the authors almost invariably state that the patient was dying of advanced heart or liver disease and that salvarsan had been given as a last resource.

At about this time, Wechselmann[18] published his little book on the pathogenesis of salvarsan fatalities, which made a profound impression in view of his extensive experience with the drug, with only one death occurring in a young girl who had received 0.1 and 0.2 gm. of salvarsan. Wechselmann attributed this fatality to defective elimination, the result of preceding mercurial treatment. According to his analysis, only the smallest number of fatalities could be attributed directly to salvarsan and, while he was unable to offer a uniform explanation fitting all cases, he was of the opinion that the simultaneous or preceding administration of mercury was particularly dangerous from the standpoint of injury of the kidneys resulting in deficient elimination of the drug with the consequent production of the highly toxic oxidation products of which "arsenoxid" was the type.

The most valuable recent analysis of fatalities has been published by Meirowsky,[19] acting as secretary of a committee appointed by the Allgemeiner aerztlicher Verein of Cologne to investigate the subject. This report deals with 13,000 injections of old salvarsan, 40,954 of sodium salvarsan, 171,826 of neosalvarsan and 64,500 of silver salvarsan. The chances of fatal reactions were estimated as follows:

The Committee found that when the dose of neosalvarsan exceeded 0.6 gm. the deaths rose to 1 to 3000 and hence recommended that 0.6 gm. be the maximum dose.

1 in 13,000 injections of old salvarsan
1 in 20,000 injections of sodium salvarsan
1 in 162,800 injections of neosalvarsan

One case of jaundice was recorded for each 5000 injections: one in 2000 for old salvarsan and one in 6000 for neosalvarsan. Injury of the skin was recorded in 48 cases, including five that terminated fatally. Arndt[20] states that during 1921 there were 14,991 injections of salvarsan given in his clinic to 1913 patients. Twelve deaths occurred or about 1 to every 1250 injections: 1 of hemorrhagic encephalitis, 1 of myelitis, 3 of dermatitis and 7 of acute or subacute yellow atrophy of the liver. Certainly this was a much higher death rate than usual and suggestive of the use of a rather highly toxic brand of salvarsan.

These reports show, however, that neoarsphenamin is much safer than arsphenamin, a conclusion well substantiated by experience. In the United States the incidence of fatalities has dropped since 1918, owing to the greater use of neoarsphenamin. As previously stated, the wonder is that the incidence of fatalities from injections of arsphenamin and its substitutes is as low as reported, there being approximately 600 recorded cases, although it is to be kept in mind that not all fatalities have been reported by any means, and especially those due to errors in administration. I personally know of several due to the accidental administration of concentrated acid solutions of arsphenamin given in mistake for neoarsphenamin and sulpharsphenamin and have heard of additional cases, that have not been recorded. But, excluding fatalities due to this grievous error, the incidence is indeed very low and apparently most have been due to two factors, namely, (*a*) errors in the technic of administration and (*b*) an unwise disregard of contraindications; both subjects are of extreme practical importance and are discussed with more detail in following chapters. Not infrequently the suggestion is made that fatalities are hushed up by arsphenamin enthusiasts and that Ehrlich and other German physicians have made strenuous and persistent efforts to blame these deaths upon everything but the drug and particularly upon errors in technic of administration, pathological states of the patients, etc., but I do not agree with statements of this sort. It is quite clear, when one refers to an "injection of arsphenamin," that this loose expression implies more than the drug alone, since it consists of other possible toxic factors as the water employed, sodium chlorid, sodium hydrate, bacteria or their disintegration products, etc.

Comparative Rapidity of Fatal Toxic Reactions from Arsphenamin and Neoarsphenamin in Rats.—As previously stated, the toxic effects of arsphenamin upon the rat are much more rapid than in the case of neoarsphenamin. For this reason, the Hygienic Laboratory has ruled that in the official toxicity tests the results may be evaluated at the end of forty-eight hours after injection in the case of arsphenamin, but not until the end of seven days in the case of neoarsphenamin. In addition to the figures which I have already quoted above from an analysis of my records of toxicity tests with rats, the following percentages of fatal reactions after the intravenous injection of 201 different lots of arsphenamin in dose of 0.100 gm. per kilo (1042 rats), and of 280 different lots of neoarsphenamin in dose of 0.200 gm. per kilo (1585 rats), may not be without some intreest in relation to the comparative rapidity of fatal reactions after the injection of these compounds, the technic employed being exactly as described in Chapter II:

	Arsphenamin	Neoarsphenamin
Percentage succumbing at once	11.1	0.6
Percentage succumbing in 24 hours	15.4	3.2
Percentage succumbing in 48 hours	3.2	1.9
Percentage succumbing in 72 hours	0.5	4.2
Percentage succumbing in 5 to 7 days	2.3	17.6
Percentage succumbing in 8 to 10 days	2.3	4.4
Percentage succumbing in 11 to 14 days	2.2	2.9
Percentage surviving more than 14 days	63	65.2

In other words, from 35 to 37 per cent. of rats injected intravenously with 0.100 gm. disodium arsphenamin and 0.200 gm. neoarsphenamin per kilo succumbed within 14 days after injection, but in the case of arsphenamin approximately 30 per cent. of these deaths occurred within the first forty-eight hours after injection, whereas with neoarsphenamin only 5.7 per cent. of deaths occurred in this time; on the other hand, at the end of seven days the deaths among the rats receiving arsphenamin was 32.5 per cent. and among those receiving neoarsphenamin approximately 27.5 per cent., showing that arsphenamin is more rapidly toxic than neoarsphenamin under well controlled experimental conditions.

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CHAPTER XLV

PHYSICOCHEMICAL CAUSES OF TOXIC REACTIONS PRODUCED BY ARSPHENAMIN, NEOARSPHENAMIN AND OTHER ORGANIC ARSENICAL COMPOUNDS

With this brief survey and summary of the numerous possible causes and clinical varieties of the toxic reactions that may follow the administration of arsphenamin neoarsphenamin and other organic arsenical compounds in the treatment of syphilis and other diseases, it is now advisable to discuss with more detail the nature and mechanism of the causes insofar as present knowledge permits, because a good understanding of these enables the physician to treat toxic reactions more successfully and what is more important, to exercise proper precautions for their prevention. Since the immediate and early untoward effects are frequently due to physicochemical factors and changes in solutions of the drugs injected intravenously, it is logical to discuss these first.

Physical Chemistry of Arsphenamin and Neoarsphenamin in Relation to Toxic Reactions.—It is now generally believed that solutions of arsphenamin hydrochlorid (acid solutions) as well as alkaline solutions of the compound (disodium salt) and solutions of neoarsphenamin possess well-marked colloidal properties and that various physical conditions may influence the

colloidal or physical chemistry of these solutions. In 1914 Fleig[1] pointed out that the physical state of the solutions, in particular their clearness, varied with the amount of sodium hydroxid and sodium chlorid present, an insufficient amount of the former with an excess of the latter producing a distinct turbidity, which he explained as being due to a precipitation of the arsphenamin base. Clinical experience and animal toxicity tests have shown me that such solutions of either disodium arsphenamin or neoarsphenamin are of increased toxicity and much more likely to produce early "nitritoid" reactions.

Hugo Bauer[2] investigated the colloidal properties of silver and sodium salvarsans, collargol and atoxyl, and concluded that they belong to the class of semi-colloids, the sizes of the particles being such as represent the dividing line between the colloids and crystalloids. Silver arsphenamin, he claims, is a chemically uniform substance with the silver existing in a complex combination with arsphenamin and not merely as mechanically admixed colloid.

Raiziss and Gavron,[3] after thoroughly investigating the colloidal properties of arsphenamin and neoarsphenamin, reached the conclusion that solutions of both in water had colloidal properties, the former behaving like a reversible colloid since when once precipitated from solution, it can be redissolved into a colloidal solution in contradistinction to the proteins and various colloidal metals which are irreversible colloids. They also observed that in solutions of silver and gold arsphenamin the metals did not diffuse as stated by Bauer, which indicates that either the metals are combined to the arsenic or nitrogen and split off during diffusion, or, as they believe, that these compounds are not homogenous combinations but mixtures of sodium arsphenamin and colloidal metals.

The investigations of Klemensiewicz[4] upon the viscosity of aqueous solutions of arsphenamin have likewise indicated their colloidal nature. He observed that the viscosity increased with the concentration of the solutions and reached the conclusion that arsphenamin is to be classed among the "lyophiles" or "emulsoids" and that the increase in viscosity is due to polymerization of the compound. Recently Voegtlin, Johnson and Dyer[5] have shown that this question of viscosity of acid and alkaline solutions of arsphenamin bears a relation to toxicity for rats and in searching for an explanation of the differences in viscosity in relation to the toxicity of arsphenamins prepared by different methods by manufacturers, concluded that the method of precipitation employed (by ether from a methyl alcohol solution of the drug or fairly concentrated hydrochloric acid from an aqueous solution) largely determined the solubility, viscosity and toxicity of arsphenamin hydrochlorid, the method of precipitation of the drug by ether from methyl alcohol solution yielding products more readily soluble and of lower viscosity than lots prepared by precipitation by hydrochloric acid. At any rate these investigations have suggested that important physico-chemical changes may occur in solutions of arsphenamin without involving any oxidative changes which bear an important relation to toxicity. Voegtlin and his associates have suggested that the decrease in toxicity of solutions accompanying the decrease in viscosity are due essentially to dispersion of the particles in solution, thereby reducing the power of such solutions to enter into reactions with the colloidal constituents of the blood. In this respect they believe that arsphenamin acts like an amphoteric colloid and the results have indicated from a different angle that the toxicity of arsphenamin is subject to great changes according to the manner in which the drug is prepared for injection; the results have also emphasized the necessity for preparing solutions in an absolutely uniform manner for biological testing as previously discussed in Chapter II, as well as the importance of technic in the administration of the drug by intravenous injection to human beings, which is discussed with more detail in Chapter XLVIII.

While the colloidal state of solutions of arsphenamin may, therefore, have a direct bearing upon their toxicity, it would appear that even more important changes in the physical colloidal state of these may occur after intravenous injection and that additional toxic agencies may be brought into play by a disturbance of the colloidal equilibrium of the plasma. For example, Danysz[6] believes that a short time after the intravenous injection of solutions of the disodium salt of arsphenamin that the sodium combines with the free carbonic acid of the plasma, forming soluble carbonate while leaving a precipitate of the insoluble base and that the remainder of the mono or disodium salt, combining with the calcium phosphates, also produces an insoluble compound. I shall later in this chapter refer again to this theory, but here it may be mentioned that Dale[7] points out that alkaline solutions of

arsphenamin are practically insoluble at the pH of the blood, and must therefore circulate in a colloidal form, aggregation of the particles being prevented by the plasma proteins. Furthermore, the Salvarsan Committee of the British Medical Research Committee have expressed a similar view in these words: "There is a good deal of evidence in support of the view that the varying toxicity of different samples of '606' is related to small differences in the physical properties of the product, which determine the state of physical dispersion in which the free base comes out of solution when the alkaline solution is injected intravenously." What the evidence for this view is, was not very fully stated. An instance is cited, however, in which samples of arsphenamin dissolved in tap-water caused symptoms of vasomotor disturbances, whereas, when dissolved in distilled water no symptoms resulted, while other samples could be dissolved in tap-water with impunity. The Committee concluded: "It would be very difficult to reconcile such an experience with the view that abnormal toxicity depends on the presence of an undue proportion of more toxic by-products," which leads to the inference that physical factors were held chiefly responsible for the varying toxicity.

Hunt[8] has also observed, with certain lots of arsphenamin, phenomena which strongly supports the idea that the physical condition (colloidal?) of the solution or the presence of a very labile toxic compound may at times account for untoward reactions. He has found that some preparations of arsphenamin are very toxic when the solutions are prepared at ordinary room temperature, and that the toxicity is greatly reduced by moderate heating or by allowing the solutions to stand for a time at room temperature; Hunt suggests that these changes in toxicity may be due to a simultaneous change in the physical state of the solution.

It is practically certain that hemolysis and agglutination of erythrocytes by solutions of arsphenamin are colloidal phenomena and when occurring intravascularly may lead to the production of toxic reactions largely due to altered proteins and pulmonary embolism. In other words, the colloidal nature of arsphenamin and neoarsphenamin may result in the production of toxic reactions *per se* when injected intravenously or, more importantly, bring about the production of disturbances in the colloidal equilibrium of the plasma and under certain circumstances such physical (colloidal?) reactions as intravascular agglutination and precipitation, with consequent toxic reactions.

However, before leaving the present subject brief reference may be made to the investigations of Elvove and Clark[8a] upon the acid-base equilibria of arsphenamin solutions, and especially in relation to the alkalinization of arsphenamin solutions for administration. Obvious errors were found in the usual indicator methods of measuring pH values, but hydrogen electrode measurements gave reproducible and better data which were confirmed by the rate of hydrolysis of methyl acetate. This method however, is not adaptable for clinical use, although the authors believe that alizarin yellow R may be used as an indicator for checking correct alkalinization of arsphenamin solutions if there are reasons for suspecting that the normal solution of sodium hydroxid employed for this purpose may be defective. Hunter and Patrick[8b] have also studied the isoelectric point by conductivity measurements in relation to the physical and colloidal nature of arsphenamin, and offer an explanation for the abnormalities found in conductivity measurements in different solutions which is based upon McBains' conception of an ionic micelle found in solutions of colloidal electrolytes and offering a possible explanation for the differences between the toxicities of acid and alkaline solutions.

Hemolysis by the Organic Arsenical Compounds in Relation to Toxic Reactions.—Among the possible physical causes for toxic reactions following the intravenous injection of arsphenamin and its substitutes, that of intravascular hemolysis may be discussed, not only because the drugs themselves may be hemolytic under certain conditions but also because of the possibility of hemolysis by hypotonic solutions if water is employed for preparing the solutions and, in the case of arsphenamin, by the presence of an excess of acids or alkali as well.

In 1920, Schamberg, Raiziss, Weiss and myself[9] briefly reported that both acid and alkaline solutions of arsphenamin in water or saline solution were hemolytic *in vitro*, and especially acid solutions. We also observed that 2- to 4-per cent. solutions of neoarsphenamin in water or saline solution were not ordinarily hemolytic *in vitro* although 0.5 per cent. solutions in water were hemolytic due to the hypotonic solution. Later Miss Yagle and myself[10] studied the hemolytic effects of arsphenamin and neoarsphenamin for *washed* erythrocytes *in vitro* more extensively and concluded that hemolysis by solutions of the disodium salt of arsphenamin was due more to solutions and excess of alkali than to the drug itself. In the case of neoarsphenamin, we found that solutions in saline were not ordinarily hemolytic although dilute solutions in water may be hemolytic due to the effects of hypotonic solutions. Subsequent investigations by Miss Richter and myself have shown that arsphenamin and sulpharsphenamin, are directly hemolytic *in vitro* for washed corpuscles although much less so for corpuscles in the presence of serum, as first shown by Crabtree[11] and amply confirmed by our experiments. Naturally the fact that arsphenamin and its substitutes are much less hemolytic in the presence of the plasma proteins *in vitro* greatly reduces the significance to be placed upon intravascular hemolysis as a cause for toxic reactions, but the protection may not be complete under certain conditions and the phenomenon both *in vitro* and *in vivo* is worthy of brief discussion in relation to toxic reactions.

Hemolytic Activity of Arsphenamin in Vitro.—(a) Solutions of arsphenamin in water without neutralization (*acid solutions;* pH about 4.6) are highly hemolytic for washed corpuscles and to a lesser extent for whole blood; in this case hemolysis may be ascribed to the acids, water and drug. Similar solutions in saline are much less hemolytic and especially for whole blood, because in the latter the plasma affords considerable protection of the corpuscles against the hemolytic activity of both acids and the drug itself and hemolysis by hypotonic solutions does not enter into consideration under these conditions.

(b) *Solutions of the monosodium salt of arsphenamin* (solutions neutralized with sodium hydroxid just to the point of neutralization and clearing) in *water* are hemolytic for *washed* corpuscles due primarily to the hemolytic activity of the hypotonic solution and secondarily to the drug. But hemolysis for *whole blood* is very much less than for washed corpuscles, because the plasma proteins protect the corpuscles against hemolysis by the drug as shown by Crabtree, and the salts of the plasma establish sufficient tonicity unless the proportions of solution to blood are excessive.

It may be said, therefore, that when a solution of the monosodium salt of arsphenamin in isotonic saline is administered by slow intravenous injection, permitting immediate dilution of the solution in the blood, that hemolysis is negligible or does not occur at all; certainly there is no reason for suspecting hemolysis under these conditions when saline solution is employed because the solution is isotonic, the acids are neutralized, there is no excess of sodium hydroxid and the corpuscles are protected by the plasma against the feeble hemolytic activity of the drug itself and traces of alkali. For these reasons solutions of the monosodium salt of arsphenamin are the least hemolytic of all and from this standpoint are the most desirable for intravenous injection.

(c) Solutions of the *disodium salt of arsphenamin* (usually containing about one-third excess of sodium hydroxid or about 0.9 c.c. of a normal solution per 0.1 gm. of drug and possessing a pH of about 9.9) are slightly more hemolytic for both washed corpuscles and whole blood than the solutions of monosodium arsphenamin, due to the excess of alkali. Dilute solutions in water (0.1 gm. per 20 c.c.) are slightly more hemolytic than similar solutions in saline.

Glucose affords no protection against hemolysis of human corpuscles *in vitro;* solutions of disodium arsphenamin in saline are just as hemolytic and solutions of the same in 1- to 5-per cent. solutions of glucose in saline.

Different lots and brands of arsphenamin are quite uniform in hemolytic activity when the solutions are prepared and tested in exactly the same manner. The corpuscles of different human beings may, however, show widely varying resistance to hemolysis by arsphenamin, but injections of arsphenamin and neoarsphenamin in the treatment of syphilis have no marked or constant effect in either increasing or decreasing resistance to hemolysis *in vitro*. These observations have been of special interest in relation to the occurrence of jaundice during treatment with arsphenamin and likewise in the treatment of non syphilitic anemia with arsphenamin to which further reference will be later made. If arsphenamin injections have any influence at all upon resistance of the corpuscles to hemolysis *in vitro*, the experiments made by Miss Richter and myself with syphilitic individuals under treatment, and with rabbits injected with therapeutic doses, indicate that after three to five doses at weekly intervals resistance is slightly lowered, followed later by restoration to the same resistance observed before treatment was given or possibly a very slightly increased resistance.

Crabtree has also made the interesting observation that the hemolytic activity of different samples of arsphenamin for washed corpuscles varied in a general way with their toxicity for rats, when the latter depended upon variations in the conditions of reduction of the nitro to the amino-group in the preparation of the drug. The hemolytic activity of different samples varied roughly, as did their toxicity for rats, but this was not constant or sensitive enough to be used as a substitute for the rat test for determining the toxicity of arsphenamin. Crabtree has also found that, on standing or shaking alkaline solutions of arsphenamin, the hemolytic activity for washed erythrocytes is decreased and may disappear altogether; also that solutions of m-amino-p-hydroxy-phenylarsenious oxid ("arsenoxid") are non-hemolytic; in other words the increased toxicity of "oxidized" solutions of arsphenamin are not to be ascribed to an increase of hemolytic activity. Crabtree also observed that warming a solution of disodium arsphenamin to 55°C. decreased its hemolytic activity and this is quite in agreement with the fact that solutions of arsphenamin prepared in hot water are not more toxic than solutions prepared with cold water. I have repeated these experiments and found essentially similar results; also that "oxidation" of solutions of disodium arsphenamin results in an even greater loss of agglutinative activity for human erythrocytes. Even the mere matter of allowing a solution to stand a few hours at room temperature may result in reducing agglutination without any change in hemolytic activity.

The exact mechanism of hemolysis by arsphenamin is not known; doubtless profound intracellular colloidal reactions occur resulting in a rupture of the cell with liberation of hemoglobin and solution of stroma. As shown by Crabtree, arsphenamin unites with washed corpuscles in the test-tube and if the solution is insufficient for producing hemolysis (1:5000) the corpuscles may be removed by centrifuging and when re-suspended in saline are found to possess an increased resistance to the hemolytic action of water or hypotonic saline solutions, as Gunn[12] has described for solutions of sodium arsenite and sodium arsenate. This increased resistance, however, only delays hemolysis and is not absolute and no increase of resistance was found to serum hemolytis by specific hemolysis and guinea-pig complement.

Of interest in connection with this antihemolytic activity of weak solutions of arsphenamin is the question of whether or not human corpuscles acquire an increased resistance to arsphenamin after preliminary exposure to weak solutions of the drug in relation to the possibility of changes of this kind occurring during the treatment of syphilis with intravenous injections of arsphenamin. I have observed that when washed human corpuscles are exposed to 1:5000 solutions in saline for an hour at 37°C. followed by centrifuging and resuspension in saline, that the "sensitized" corpuscles are, however, not any more resistant to hemolysis by arsphenamin than control suspensions. Similar effects were observed in relation to the agglutination of human corpuscles by arsphenamin, to which reference will be later made.

As previously stated, the presence of serum tends to protect corpuscles against the hemolytic activity of saline solutions and the disodium salt of arsphenamin; I have observed similar effects with other hydrophil colloids

as gelatin and acacia. The mechanism of this protection by colloids is not known but I surmise that it is the same as advanced by Oliver and his associates in explanation of the mechanism of protection of erythrocytes by these substances against the agglutinative activity of arsphenamin. According to this theory, arsphenamin is "bound" by the protective colloid as the proteins of the plasma and especially by the globulins, gelatin, acacia, etc., preventing the union of arsphenamin with the corpuscles as well as preventing agglutination. I believe that the same theory may be accepted in explanation of the protection afforded by the plasma proteins and gelatin against hemolysis *in vitro*.

Technic of Administration of Arsphenamin in Relation to Hemolysis.—It is apparent that the possibility of early toxic reactions due to intravascular hemolysis may be readily influenced by technical factors in the method of administration. For example, if a solution is infused rapidly it is entirely likely that the blood in the vein may be so diluted at the site of injection that little or no protection is afforded the erythrocytes mixing with the solution against hemolysis by the drug or by the excess of alkali and hypotonic solution, if water instead of saline is employed. The injection of a concentrated solution and especially rapid injection would tend to produce similar conditions and greatly increase the degree of hemolysis. It is well known that rapid injection and especially rapid injection of concentrated solutions, increase the percentage of toxic reactions and it may well be that the factor of intravascular hemolysis is involved along with other physical phenomena as agglutination, precipitation of proteins and the like.

When arsphenamin is administered by intravenous injection, the solution should be isotonic by preparation with fresh saline solution; it should be dilute (as 0.1 gm. per 20 c.c.) as recommended by the Hygienic Laboratory; too great excess of alkali should be avoided and the solution should be injected slowly in order to minimize the chances of intravascular hemolysis.

Hemolytic Activity of Neoarsphenamin in Relation to Technic of Administration and Toxic Reactions.—Neoarsphenamin is of much less importance than arsphenamin from the standpoint of toxic reactions to be ascribed to intravascular hemolysis. As manufactured at present, solutions are usually neutral in reaction and the drug itself does not produce hemolysis of *whole blood* although concentrated solutions (20- to 40-per cent.) may be feebly hemolytic for washed erythrocytes; in other words, neoarsphenamin itself is very much less hemolytic than arsphenamin and it is entirely likely that *in vivo* the plasma proteins effectually protect the corpuscles against its hemolytic action as they do in the test tube. *Dilute solutions of neoarsphenamin in water, however, may be hemolytic* because of the hypotonic state of the solution as observed by Miss Yagle and myself.

Hemolysis after the administration of neoarsphenamin is, therefore, almost entirely a matter of technic of administration. When the usual dose of 0.6 to 0.9 gm. is dissolved in 5 to 15 c.c. of distilled water the solution is not hemolytic for whole blood and its administration does not produce hemolysis. When 0.6 to 0.9 gm. are dissolved in 50 c.c. or more of water, the solution is hypotonic and hemolytic; the more water used, the greater the degree of hemolytic activity. When 0.6 to 0.9 gm. are dissolved in 1 c.c. of water the solution may be hemolytic, but is not apt to be for the whole blood *in vivo* if the solution is slowly injected. From the standpoint of administration, therefore, the usual dose of 0.6 to 0.9 gm. neoarsphenamin should be dissolved in 5 to 15 c.c. if distilled water is employed; if 50 c.c. or more fluid is used preference should be given to saline solution.

Hemolytic Activity of Sulpharsphenamin in Relation to Administration and Toxicity.—Since sulpharsphenamin is sometimes administered by intravenous, as well as by subcutaneous and intramuscular injection, the question of its hemolytic properties likewise becomes a matter of interest in relation to toxic reactions. In my experience, solutions of this arsenical in *saline* are practically without hemolytic activity for whole blood. Concentrated solutions prepared by dissolving 0.5 gm. in 5 c.c. of saline solution may prove slightly hemolytic for washed erythrocytes with the production of methemoglobin when the mixtures are allowed to stand for an hour or more at 37°C., but with whole blood these changes do not ordinarily occur owing to the protection afforded by the plasma or serum. *Solutions in water, however, are slightly hemolytic* for both washed erythrocytes and whole blood, due primarily to the effects of hypotonic solutions.

In practice, the administration of 0.4 to 0.6 gm. of sulpharsphenamin in 5 to 15 c.c. of sterile distilled *water* by intravenous injection, probably produces a slight degree of hemolysis of erythrocytes at the point of injection where the solution comes in contact with the cells before dilution with the blood to the point when tonicity results; when this drug, however, is dissolved in *saline solution* hemolysis probably does not occur at all.

Hemolytic Activity of Atoxyl, Tryparsamid and Other Organic Arsenical Compounds.—In my experience solutions of *atoxyl and tryparsamid in saline solution* are not hemolytic for either whole blood or washed erythrocytes. *Solutions in water may be slightly hemolytic* and more so for washed erythrocytes than for whole blood, due to hypotonicity. As previously mentioned, Crabtree found that solutions of "arsenoxid" in saline were non hemolytic for washed sheep corpuscles.

In searching for the cause of the hemolytic activity of arsphenamin, Crabtree[13] examined solutions in saline of the sodium salts of a number of substituted phenyl arsenic acids and two polyarsenids against washed sheep corpuscles with negative results and his study has shown that the hemolytic activity of the arsenical compounds increased in direct proportion to the number of amino-groups introduced, as has been previously mentioned and discussed.

Rôle of Hemolysis in the Production of Toxic Reactions.—It is apparent that with a few simple precautions in technic the hemolytic activity of arsphenamin, neoarsphenamin and other organic arsenical compounds *in vivo* can be largely or entirely prevented. Arsphenamin is most likely to produce hemolysis, but by using a saline solution instead of water, avoiding an excess of sodium hydroxid and injecting slowly, hemolysis can be greatly reduced since the plasma proteins afford a considerable degree of protection to the erythrocytes against hemolysis by alkali and the drug.

It is highly probable, when hemolysis occurs, that most takes place immediately after injection as the infused solution comes in contact with erythrocytes. As the solution becomes diluted with blood in the veins, hemolysis becomes progressively less and unimportant. Furthermore, it appears that the oxidized products of arsphenamin and its substitutes are non-hemolytic and delayed intravascular hemolysis probably does not enter into the production of the late toxic effects of these arsenical compounds.

The symptoms of toxic action which one may ascribe to hemolysis are those which resemble "protein shock," such as fever, sweating and nausea developing within one to three hours after injection. As previously mentioned, it is to be admitted that very small amounts of hemolysis may be sufficient for these effects and that susceptibility of erythrocytes to hemolysis may vary in different individuals and that concentration of the solutions and

rapidity of injection may be sufficient to break up enough cells to yield reactions of this kind and degree.

Intravascular Agglutination by the Organic Arsenical Compounds in Relation to Toxic Reactions.—Of even more interest and importance than intravascular hemolysis in relation to the mechanism of toxic reactions by arsphenamin and its substitutes, is the possibility of intravascular agglutination of erythrocytes by these medicaments. Early investigations by Meissner,[14] v. Marscholkó and Veszprémi[15] and Schindler[16] of cases of fatal arsphenamin poisoning emphasized the importance of multiple emboli and thrombi, especially in the lungs, which were ascribed to the action of arsenic upon the blood-vessels. Lucke and myself[17] regularly noted peculiar "brassy" thrombi of conglutinated erythrocytes in sections of the lungs and other organs of rabbits and rats after intravenous injection of solutions of arsphenamin and to much lesser extent after neoarsphenamin in our series of studies upon the pathological tissue changes produced by these compounds; we ascribed these effects primarily to the fixing fluids employed for the histological examination of the tissues, but I now believe that in some instances agglutination of the corpuscles *in vivo* were responsible rather than conglutination by the fixatives. These and other investigations about to be discussed have indicated that under certain conditions the administration of arsphenamin may cause intravascular agglutination of erythrocytes and produce definite lesions and symptoms of toxic action largely due to such physical factors as thrombosis and embolism.

Agglutination of Erythrocytes by Arsphenamin in Vitro.—In 1920, while Karsner and Hanzlik[18] were investigating the production of "anaphylactoid" phenomena in guinea-pigs by the intravenous injection of agar, acacia, etc., it was found that the injection of therapeutic sized doses of solutions of disodium arsphenamin and neoarsphenamin sometimes produced respiratory distress with pulmonary congestion and hemorrhages but pulmonary distention with emboli and thrombosis were generally absent and the results were ascribed primarily to the effects of arsenic upon the blood-vessels and circulation.

Later Karsner and Hanzlik[19] observed that solutions of arsphenamin and neoarsphenamin agglutinated *washed* human, cat and guinea-pig erythrocytes in the test-tube and the intravenous injections of the former was believed to produce agglutination and thrombosis in the pulmonary vessels.

Oliver and Douglas[20] subsequently confirmed the observations that solutions of disodium arsphenamin agglutinated *washed* human erythrocytes and the corpuscles of the lower animals in the test-tube. These investigators found that the corpuscles first absorb the drug, but that agglutination does not result unless an electrolyte is present which results in producing a physical change in the degree of dispersion of the arsphenamin in solution. They also observed that solutions of arsphenamin exposed to air lose in agglutinating power, which is interesting in view of the findings of Crabtree referred to above, to the effect that oxidized solutions lose in hemolytic activity.

Subsequently Oliver and Yamada[21] found that the agglutination of washed erythrocytes by arsphenamin *in vitro* may be largely prevented by the presence of serum or other hydrophilic colloids which naturally reduce the significance to be placed upon the possibility of agglutination *in vivo* as a cause of toxic reactions. Inhibition of agglutination of washed corpuscles in the test-tube by serum and other colloids was believed to be due to an interference of union of arsphenamin with the corpuscles as well as to interference with the act on of the electrolyte sodium chlorid. Of the colloids studied, namely, gelatin, serum, gum arabic, egg albumin and starch, most protection was afforded by gelatin, serum and gum arabic named in the order of efficiency. In a subsequent study of the mechanism of protection of erythrocytes against the agglutinating activity of arsphenamin by serum and other colloids, Oliver and Douglas[22] found that the plasma proteins, especially the globulins, unite with the arsphenamin and thereby prevent the binding of the drug by the corpuscles. This was believed to be the physiological method of protection against agglutination *in vivo* following the intravenous injection of therapeutic doses. My investigations conducted with the assistance of Miss Richter, have amply confirmed these observations of Oliver and his associates and may be briefly summarized as follows:

(1) For washed human erythrocytes solutions of the *disodium salt of arsphenamin* caused slight but definite agglutination in final dilutions as high as 1:3000 to 1:25000; this means

that 0.0003 to 0.0004 gm. of arsphenamin produced some agglutination when added to 1 c.c. of a 2-per cent. suspension of washed corpuscles.

(2) Different lots of arsphenamin were found to vary to a slight degree in agglutinating activity. All agglutinated in final dilution of 1:3000; an occasional lot produced some microscopic agglutination in final dilutions as high as 1:100,000.

(3) *Acid solutions of arsphenamin* caused greater agglutination of washed erythrocytes than solutions of the monosodium and disodium salts.

(4) As previously stated, Crabtree observed that "oxidized" solutions of disodium arsphenamin were less hemolytic than fresh solutions. Similar results were observed by us and we have also found that the loss in agglutinative properties is even more marked.

(5) Heating and shaking solutions of the disodium salt of arsphenamin has a tendency to reduce agglutinating activity.

(6) No noteworthy differences were found in the agglutination of erythrocytes of different healthy non-syphilitic human beings by the same lot of arsphenamin.

(7) The corpuscles of syphilitic individuals under treatment with arsphenamin and neoarsphenamin occasionally showed a slightly increased susceptibility to agglutination and especially after three or more injections had been given.

(8) Glucose increased the susceptibility of washed human erythrocytes to agglutination by arsphenamin. For example, solutions of disodium arsphenamin in saline agglutinated 1:3000 to 1:6000; solutions of the same arsphenamin in 5-per cent. solutions of glucose in saline agglutinated the same erythrocytes in final dilutions as high as 1:200,000.

The protection afforded corpuscles by hydrophil colloids as serum or plasma, gelatin, acacia, etc., against agglutination by arsphenamin is due, according to Oliver, to a binding of the arsphenamin by the colloid which thereby protects the corpuscles. The amount of arsphenamin bound was found to be markedly influenced by the H-ion concentration of the medium in which the reaction occurred. With serum or plasma it is believed that the globulins are the active protective agents and the degree of protection afforded by these is quite marked. The protection afforded by gelatin is also quite marked; for example, solutions of disodium arsphenamin in saline solution caused some agglutination of washed human corpuscles in final dilutions as low as 1:600. *Acacia* is much less protective of corpuscles against agglutination and hemolysis by disodium arsphenamin. In the same experiments 2-per cent. solutions afforded no protection against hemolysis by 1:400 solutions but $\frac{1}{8}$ per cent. solutions protected against agglutination.

Agglutination of Erythrocytes by Arsphenamin in Vivo in Relation to Toxic Reactions.—Since the plasma proteins so effectually protect erythrocytes against agglutination by arsphenamin *in vitro*, one may naturally conclude that intravascular agglutination is not produced by the amounts ordinarily administered at one time by intravenous injection in the treatment of syphilis. But it is possible that the rapid injection of solutions of arsphenamin and especially of concentrated solutions may so dilute the blood in the vein at the site of injection as to "saturate" the plasma proteins and thereby reduce the protection afforded by the plasma so that the excess of "unbound" arsphenamin may unite with corpuscles and produce some agglutination; certainly this possibility exists when acid solutions are administered by mistake and evidence is at hand to show that part of the severe toxic reactions following this technical error are to be ascribed to intravascular agglutination.

In a study by Lucke and myself[23] of the histo-pathological changes produced in rabbits by the intravenous injection of arsphenamin, emboli were commonly observed in many of the internal organs and especially in the lungs. These were especially conspicuous after the intravenous injection of fatal amounts of acid solutions of arsphenamin and likewise after the injection of large and lethal amounts of solutions of the disodium salt. But even after repeated intravenous injections of rats and rabbits with the disodium salt in dose of 0.010 gm. per kilo of weight which corresponds to 0.6 gm. per 60 kilos or approximately the dose for adult human beings, emboli and thrombi of erythrocytes were sometimes found not only in the lungs but likewise in the brain, liver, spleen and other internal organs. Lucke and I at the time thought that these intravascular masses were composed of precipitates and partially hemolyzed erythrocytes and that in part they were produced after death by the fixing fluids employed. But since then the work of Oliver and

Yamada[24] has shown that in the rabbit solutions of the disodium salt of arsphenamin may produce intravascular agglutination and that the thrombi may be masses of agglutinated cells and especially after the intravenous injection of massive doses.

These investigators examined blood from the vena cava, ear veins and perfusion fluids from the spleen and lungs of rabbits at intervals of a few minutes to six hours after intravenous injections of the drug and observed evidences of agglutination after the injection of doses varying from 0.110 to 0.420 gm. per kilo; especially striking results were observed in those animals succumbing almost immediately. Clumps of agglutinated erythrocytes were found on both sides of the vascular system, in the right side of the heart and in the left ventricle. Evidences of embolism were found in practically all organs either by direct inspection with a hand lens or microscopically after sectioning of the tissues and the symptoms produced as dyspnea, convulsions and death were likewise observed after the injection of corpuscles previously agglutinated in the test tube by arsphenamin and similar to the effects produced by oil embolism.

Essentially similar results were observed in the experiments of Miss Richter and myself employing the methods described by Oliver and Yamada. Rats injected intravenously with solutions of disodium arsphenamin in doses of 0.050 to 0.200 gm. per kilo regularly showed agglutinated masses of erythrocytes in the right auricle and inferior vena cava when examined within a few minutes after injection. Rabbits under chloral anesthesia were injected by way of the ear veins with 2-per cent. solutions of disodium arsphenamin and the blood of the external jugular vein examined at frequent intervals. After the injection of 20 c.c. corresponding to about 0.2 gm. per kilo and at the rate of about 2 c.c. per minute, agglutinated masses of corpuscles were found in the jugular blood eight minutes after injection; masses were also found in the right auricle of the heart, but not in the inferior vena cava. Similar but less marked effects followed the injection of 10 c.c. of solution corresponding to about 0.1 gm. per kilo of weight since the rabbits weighed about 1800 grams.

It is to be carefully borne in mind that these effects were produced by very large doses of disodium arsphenamin; the smallest dose namely, 0.1 gm. per kilo, being at least ten times the maximum amount ordinarily administered to human subjects at one time. With smaller doses as 0.04 to 0.01 gm. per kilo, Oliver and Yamada were unable to definitely determine if any intravascular agglutination occurred since the occasional masses of coalesced corpuscles sometimes found in the tissues by histological methods may have been produced by the fixing fluids as surmised by Lucke and myself in our earlier studies. But it cannot be said that agglutination may not occur after the intravenous injection of alkaline solutions of arsphenamin in therapeutic doses, as 0.6 gm. for an adult. So many physical factors enter into the mechanism as the concentration of plasma proteins and electrolytes, the hydrogen ion concentration of the blood, etc., that it may be possible for intravascular agglutination to occur although severe symptoms and lesions may not be produced. This is particularly true if concentrated solutions are rapidly injected and to a lesser extent by the rapid injection of dilute solutions. When acid solutions are accidentally injected intravenously, the chances of intravascular agglutination is tremendously increased and doubtless plays a part in the symptoms and lesions produced.

Granting that under certain conditions intravascular agglutination may occur after the intravenous injection of arsphenamin in the treatment of syphilis, what symptoms may be expected? If extensive agglutination is produced, as by the injection of acid solutions, the immediate effects of

pulmonary embolism may be expected with dyspnea, cough and pain in the chest followed by the development of areas of pneumonia. Similar but less severe symptoms following the injection of alkaline solutions may be likewise due to embolism of agglutinated cells as well as to the production of precipitates to be described shortly, and whenever broncho-pneumonia follows the administration of arsphenamin effects of these kinds are to be suspected as having occurred.

The effects of pulmonary embolism may also produce in part at least the dilatation of the right side of the heart with a rise in pulmonary blood pressure and a drop in the arterial pressure. Convulsions may be due to emboli in the brain, but the effects of embolism in the liver, spleen, kidneys and other organs are not demonstrable by clinical symptoms.

In other words, the only symptoms of toxic reactions that may be ascribed to intravascular agglutination are those that develop acutely. There is no reason for suspecting that later degenerative lesions and especially in the kidneys and liver are secondary effects; rather these are to be ascribed to the effects of the arsenic and are described in the succeeding chapter. As shown by Ophuls,[25] Warthin[26] and others in studies upon the effects of embolism in general, tissue degeneration results only when capillary obstruction is extreme and complicated by stasis and secondary thrombosis and such degrees of extreme embolism certainly do not occur after the injection of therapeutic doses of arsphenamin in neutral or slightly alkaline solution.

Prevention of Intravascular Agglutination and Other Toxic Reactions by Gelatin.—The question now arises, is it possible to further aid the plasma proteins in protecting the corpuscles against hemolysis and agglutination by arsphenamin by injecting a second protective colloid as gelatin or acacia? Oliver, Yamada and Kolos[27] have studied this possibility quite extensively with special reference to gelatin and have reported that the intravenous administration of disodium arsphenamin in gelatin solutions to rabbits not only affords complete protection against intravascular hemolysis and agglutination, but also affords some protection against the organotropic or chemical toxicity of arsphenamin for such organs as the liver and kidneys. Furthermore, while according to Oliver and his associates saline solutions of disodium arsphenamin may reduce the coagulability of the blood by a destructive effect upon fibrinogen this change does not occur with gelatin solutions; circulatory failure caused by reduction of blood-pressure is also prevented by gelatin by reason of the protection afforded not only against the drug itself, but also against hydroxy ions due to the alkalinity of the solutions which Jackson and Smith believe may be a factor in the circulatory changes.

The results of my experiments have given confirmation to these observations. In the first place the *toxicity of gelatin* itself is very slight. Rabbits and rats have been injected intravenously with amounts as high as 10 grams per kilo of weight without effects insofar as duration of life was concerned. Doubtless the intravenous injection of gelatin may produce some fever, leukocytosis, etc., as do other protein substances but not to the same degree as such protein substances as foreign serum, peptone, etc.

Rabbits injected intravenously with 20 c.c. of 2-per cent. solutions of disodium arsphenamin in saline corresponding approximately to 0.200 gm. of the drug per kilo, regularly show agglutination of corpuscles in the blood of the internal jugular vein, inferior vena cava, right auricle and lungs and incoagulability of the blood. The injection of the same prepared in 0.5- to 2-per cent. solutions of gelatin in saline, autoclaved for 20 minutes, was without these effects.

The maximum tolerated dose of one lot of disodium arsphenamin in saline solution for rats by intravenous injection was 0.240 gm. per kilo; with solutions in 1-per cent. gelatin in saline the dose was 0.280 gm. per kilo. Similar results were observed with other lots of more toxic arsphenamin so that I am able to subscribe to the opinion of Oliver and his colleagues that solutions of disodium arsphenamin in gelatin are slightly less toxic for rats than solutions in saline.

Influence of Gelatin upon the Trypanocidal and Spirocheticidal Activity of Arsphenamin.—Since gelatin reduces the toxicity or organotropism of arsphenamin the very important influence upon therapeutic or parasiticidal activity requires serious consideration and extensive study before an opinion may be expressed upon the practical use of gelatin solutions of arsphenamin in the treatment of syphilis.

Trypanocidal tests were conducted with white rats infected with *Tr. equiperdum* after the methods of Voegtlin and myself described in Chapter X. Solutions of different lots of arsphenamin (disodium salt) were prepared in saline and in 1-per cent. solutions of gelatin in saline previously sterilized by autoclaving for 20 minutes. The results observed with two different arsphenamins were as follows:

Arsphenamin.....	M.T.D.* 48 Hours		M.E.D.† 48 Hours		M.C.D.‡ 14 Days	
	Saline	Gelatin	Saline	Gelatin	Saline	Gelatin
Lot A.....	0.200	0.250	0.006	0.006	0.002	0.003
Lot B.....	0.230	0.250	0.010	0.008	0.002	0.004

* Maximum tolerated dose.
† Minimal effective dose (Voegtlin method).
‡ Minimal curative dose (Kolmer method).

As just stated toxicity for the host is slightly reduced, inasmuch as the maximum tolerated doses are slightly higher when the drug is administered in gelatin solution. However, trypanocidal activity or toxicity for the parasites is also reduced according to the results observed in tests conducted with my method although in duplicate tests according to the Voegtlin method, trypanocidal activity was not materially reduced; however, I am of the opinion that gelatin protects both host and parasites against the toxic action of arsphenamin and that the therapeutic activity of the drug may be slightly reduced when administered intravenously in 1-per cent. gelatin solutions.

Essentially similar results were observed in spirocheticidal tests employing rabbits infected by intratesticular injections of *Spirochaeta pallida* as described in Chapter XXI. The minimal curative dose of arsphenamin dissolved in saline for effecting complete sterilization including the lymph glands was approximately 0.012 gm. per kilo; the dose of the same drugs in 1-per cent. gelatin solution in saline was approximately 0.018 gm. per kilo so that it may be said that solutions of the disodium salt of arsphenamin in gelatin undergo slight reduction in spirocheticidal activity.

Oliver, Douglas and Kolor,[28] however, have found that solutions of disodium arsphenamin in gelatin were retained in animals at a higher concentration for a longer period than solutions in water and that they were as effective in the treatment of experimental trypanosomiasis and rabbits syphilis as the latter, although gelatin arsphenamin was somewhat less trypanocidal *in vitro*.

Agglutination of Erythrocytes by Neoarsphenamin in Vitro.—Neoarsphenamin produces very much less agglutination of washed human erythrocytes *in vitro* than arsphenamin and different lots of neoarsphenamin vary much more in agglutinating activity than the latter; in my experiments about 50 per cent. of lots of neoarsphenamin are totally devoid of this property.

Furthermore agglutination by solutions of neoarsphenamin is very irregular, that is, final dilutions of 1:3000 to 1:12000 may produce slight agglutination after water bath incubation of one hour when 1:200 to 1:2000 dilutions produce no agglutination, indicating the importance of physical factors in the process. Even when agglutination occurs the clumps are much smaller

and fewer in number than produced by arsphenamin. Washed rabbit erythrocytes are more susceptible and slight agglutination may occur in final dilutions as high as 1:100,000 with some lots of neoarsphenamin.

In the presence of serum or gelatin agglutination does not ordinarily occur at all even in final dilutions as low as 1:20. In other words, these colloids afford complete protection of corpuscles *in vitro* against agglutination by neoarsphenamin, as is likewise true of arsphenamin as described above.

Syphilis does not appear to influence the susceptibility of washed human corpuscles to agglutination by neoarsphenamin and repeated injections of the drug to human beings and rabbits have not appeared to influence susceptibility.

Agglutination of Erythrocytes by Neoarsphenamin in Vivo in Relation to Toxic Reactions.—There is no positive evidence of the occurrence of intravascular agglutination of erythrocytes after the intravenous injection of therapeutic sized doses of neoarsphenamin. Lucke and myself[29] found intravascular masses of erythrocytes in the lungs and other internal organs of rabbits and rats injected intravenously with such massive doses of neoarsphenamin as 0.2 gm. per kilo and likewise to a lesser extent after the repeated injection of therapeutic size doses as 0.02–0.03 gm. per kilo, but in some instances at least these may have been caused by the fixing fluids employed.

The intravenous injection of rabbits (ear vein) with 10 c.c. of 4-per cent. solutions at the rate of 1 to 2 c.c. per minute and corresponding to about 0.2 gm. per kilo did not produce any evidence of agglutination in blood drawn from the internal jugular vein or in the blood of the right and left auricles of the heart and inferior vena cava as observed after the intravenous injection of 0.1 gm. of disodium arsphenamin per kilo. Even the injection of 20 c.c. of a 4-per cent. solution corresponding to approximately 0.4 gm. per kilo, since the rabbits weighed about 1800 grams, was without any evidences of intravascular agglutination in tests made of blood from the parts mentioned above at intervals of one to ten minutes after the injection had been given.

It would appear, therefore, that little or no part of the toxic reactions following the intravenous injection of *clear* solutions of neoarsphenamin are to be ascribed to intravascular hemolysis and agglutination. Sometimes the solutions are cloudy and slightly acid in reaction and these may cause a greater degree of physical reactions of this sort but at the present time such compounds are only rarely encountered. With acceptable lots of neoarsphenamin it is apparent that the plasma proteins are able to protect the corpuscles against the feeble agglutinating activity of the drug even when the latter is administered in concentrated solution by intravenous injection.

Agglutination of Erythrocytes by Sulpharsphenamin and Tryparsamid. Since sulpharsphenamin and tryparsamid are frequently administered by intravenous injection in the treatment of syphilis, the question of their influence upon human erythrocytes becomes one of importance in relation to the toxic effects sometimes observed.

Sulpharsphenamin is not nearly as agglutinative for *washed* human corpuscles as arsphenamin. Solutions in water or saline in amounts of 1 c.c. of 1:50 to 1:200 usually produce slight agglutination when added to 1 c.c. of a 1-per cent. suspension of corpuscles (final dilutions 1:100 to 1:400), but not usually in higher dilutions. Different lots and brands of sulpharsphenamin vary somewhat in this property but in the presence of serum agglutination does not occur at all even in final dilutions as low as 1:10.

The intravenous injection of rabbits and rats with doses as large as 0.2 gm. per kilo have shown no evidences of intravascular agglutination in blood

removed from the jugular vein, both sides of the heart and inferior vena cava, as is usually found after the injection of 0.1 to 0.2 gm. of disodium arsphenamin per kilo of weight. In the amounts ordinarily administered to human beings, there is no reason for suspecting that intravascular agglutination occurs or if so, to an extent sufficient for the production of symptoms unless doses of 0.4 to 0.6 gm. are dissolved in 1 to 3 c.c. of water and rapidly injected under which circumstances it is conceivable that so much of the drug may be thrown into the blood at once as may "saturate" the plasma proteins and leave an excess for union with corpuscles with the production of slight agglutination in the vein at the site of injection and especially if the injection is being given in a small vein.

Solutions of tryparsamid are apparently without effect upon washed human corpuscles, solutions as concentrated as 1:5 in saline in amounts of 1 c.c. added to 1 c.c. of 2-per cent. suspension of washed cells have not produced agglutination; at least not the brand of tryparsamid which was available for my experiments.

Intravascular Coagulation of Proteins by the Organic Arsenical Compounds in Relation to Toxic Reactions.—In addition to the possibility of intravascular hemolysis and agglutination as causes of toxic reactions following the intravenous injection of the organic arsenical compounds and especially arsphenamin, the possibility of reactions due to intravascular coagulation of the plasma proteins requires consideration in relation to the mechanism of toxic reactions by arsphenamin and its substitutes. Indeed, this subject received the earliest attention of Ehrlich and others in 1910–1911 in seeking an explanation for the unfortunate fatalities following the intravenous injection of acid solutions of arsphenamin and subsequent investigations have proven that the extremely high toxicity of concentrated acid solutions is due primarily to intravascular coagulation of the plasma proteins and probably to a lesser extent to the precipitation of the drug by the inorganic salts of the blood.

Coagulation of Blood Proteins by Acid Solutions of Arsphenamin in Relation to Toxic Reactions.—Unquestionably the intravenous injection of acid solutions of arsphenamin may, under certain conditions, produce severe and even fatal toxic reactions. Hering[30] who first called attention to this danger and showed that in rabbits the lethal dose of 1-per cent. solutions is 0.004 to 0.005 gms. per kilo and from 0.010 to 0.020 gms. per kilo for dogs, expressed the opinion that the death reported by Fraerikel and Grouves[31] following the intravenous injection of 0.4 gm. of salvarsan dissolved in 15 c.c. of water was due to the effects of the acid solution instead of to hypersensitiveness to arsenic and since then numerous other fatalities have been caused by the injection of concentrated acid solutions.

Soon after the discovery of salvarsan it was found by Michaelis,[32] Miessner,[33] Schottmuller,[34] Fleig[35] and others that acid solutions produced profound changes of precipitation and coagulation when added to blood. In the United States the early investigations of Auer[36] and Joseph[37] also drew attention to this fact and the high toxicity of acid solutions when injected intravenously.

As first shown by Auer and confirmed by Joseph, Fleig and others, the degree of toxicity of acid solutions of arsphenamin for rabbits by intravenous injection varies according to their concentration. Auer found that the injection of 0.5 per cent. solutions were very toxic whereas when 0.1 per cent. solutions were injected the toxicity was reduced four or five times and Fleig observed that 1:50 solutions were about 20 times more toxic than 1:600 solutions.

In other words, the intraveous injection of dilute acid solutions of arsphenamin are much less likely to produce intravascular coagulation and precipitation than highly diluted solutions and this fact has saved the lives of not a few human beings accidentally injected with solutions of arsphenamin in water without neutralization. Joseph has observed that in the test-tube dilute solutions are less likely to coagulate blood than concentrated solutions and Shamberg, Raiziss, Weiss and myself[38] found that acid solutions of arsphenamin in a concentration of 0.25-per cent. or greater will cause coagulation and precipitation in the presence of human serum in the test-tube.

According to Fleig, the phenomenon is one of coagulation of the protein constituents and is ascribed to the action of the phenol group of the arsphenamin molecule. The hydro-

gen-ion concentration of such solutions is approximately 4.7. In addition to coagulation of the plasma proteins there occurs agglutination and hemolysis of the corpuscles as described in preceding sections of this chapter, and likewise a precipitation of the drug itself, to which reference will be later made. In my experiments with Mr. Brown, it was found that the addition of acid solutions to serum *in vitro* results in coagulation of the proteins as well as in a lesser degree to the precipitation of arsphenamin as an insoluble compound by inorganic constituents as described by Danyzs.[39] For example, in one experiment the addition of 20 c.c. of a 2-per cent. solution of arsphenamin in 0.6 per cent. saline to 20 c.c. of serum, followed by removal of the precipitate by centrifuging and analysis of the supernatant serum, showed the following results:

	Serum Albumins Per Cent.	Serum Globulins Per Cent.	Calcium Per Cent.	Phosphorus Per Cent.
Whole serum	3.56	2.38	10.5	3.2
Serum after treatment with acid solution of arsphenamin	2.31	1.65	10.9	2.8

The symptoms, produced by the intravenous injection of acid solutions of arsphenamin the lower animals and man, develop almost immediately and are chiefly respiratory as dyspnea, compression pain of the thorax, cyanosis, cough and collapse; in cases escaping an immediately fatal issue bronch-pneumonia has usually developed.

These symptoms suggest pulmonary embolism and necropsy examinations have shown that this is the case. Meissner first observed these changes in cattle injected with fatal doses of acid solutions and Joseph who first studied the lesions with particular care, observed that in rabbits and dogs precipitates were to be usually found in the blood of the right heart and lungs but not in the arterial blood (left heart). In other words, the intravenous injection of concentrated acid solutions of arsphenamin usually produce immediate physical changes of agglutination and hemolysis of corpuscles and coagulation of proteins as well as precipitation of the drug, the whole forming embolic masses which tend to be filtered out in the capillaries of the lungs followed by the development of acute pulmonary embolism, infarction and edema with the later development of broncho-pneumonia if the acute crisis is not fatal. Lucke and myself[40] found the most extensive intravascular precipitates in rabbits and rats injected with fatal doses of acid solutions and while these were largest and most prominent in the lungs, yet similar but smaller masses were found in other organs indicating the possibility of their passage through the lungs into the arterial blood or the passage at least of the drug itself with subsequent precipitation by the inorganic salt of the plasma. However, the injection of acid solutions are not necessarily fatal and may not necessarily produce symptoms since the questions of concentration and rapidity of injection are of great importance in this connection.

Coagulation of Blood Proteins by Alkaline Solutions of Arsphenamin in Relation to Toxic Reactions.—As stated in preceding sections of this chapter, solutions of arsphenamin neutralized to the point of clearing with sodium hydroxid (monosodium salt) and alkaline solutions containing about one-third excess of alkali (disodium salt) may produce some intravascular agglutination and hemolysis if the dose, concentration and rapidity of injection are sufficient for overcoming the protection afforded by the plasma proteins. But the question now arises, are such solutions capable of coagulating or precipitating the proteins of the plasma *in vivo* and in the test-tube?

Joseph, in the experiments referred to above, saw no evidences of intravascular precipitation or coagulation when dogs and rabbits were injected with large doses of arsphenamin in 1:200 and 1:500 alkaline solutions and concluded that arsphenamin as ordinarily administered by intravenous injection in neutral or alkaline solution does not produce intravascular coagulation as occurs so readily when acid solutions of similar concentration are injected. Fleig likewise found in his experiments upon the toxicity of salvarsan that solutions of the monosodium and disodium salts produced no gross changes when added to blood *in vitro* as were produced by acid solutions. Berman,[41] however, stated a few years ago that alkaline solutions of arsphenamin may coagulate the proteins of the serum of some cases of syphilis showing an increased protein content of the blood and developing immediate reactions of flushing, dyspnea, precordial pain and cough ("nitritoid crisis") after intravenous injections.

As stated in Chapter XLIII, it is true that in some cases of syphilis there occurs an increase of the serum globulins as first described by Rowe[42] employing the refractometric method of Robertson.[43] Earlier studies by Winternitz[44] and Elias, Neubauer, Porges and

Salomon[45] had shown this increase of globulin in syphilis and in my laboratory the investigations of Tokuda[46] amply confirmed the observations of Rowe. But even though in syphilis there may be this increase of serum globulins, does the intravenous injection of alkaline solutions of arsphenamin cause any coagulation? Schamberg, Raiziss, Weiss and myself[47] found that, when solutions of the monosodium salt of arsphenamin were added to serum *in vitro*, that a slight precipiation occurs with the first few drops, but that this precipitate disappears with the addition of alkali and that solutions of the disodium salt produced no precipitation at all. In a later study by Schamberg, Tokuda and myself,[48] further evidence was obtained on the importance of the amount of alkali present in the solution of arsphenamin upon precipitation *in vitro*. Practically always solutions neutralized according to Ehrlich's method to the point of clearing (monosodium salt) produced some precipitation when added to serum *in vitro*, but the addition of a trace more of sodium hydroxid dissolved the precipitate and for this reason we believed that the precipitate was of the drug rather than a coagulation of proteins. In our investigation the serums of individuals yielding no toxic reactions after intravenous injections of arsphenamin produced just as much precipitation in the test-tube as the serums of those that showed toxic reactions after injections of arsphenamin or neoarsphenamin.

It would appear, therefore, that the intravenous injection of neutralized or alkaline solutions of arsphenamin do not coagulate the proteins of the blood; it is possible that the injection of the monosodium salt in concentrated solution may have this effect, but this is not at all likely when the disodium salt is injected even in concentrated solutions. Under these circumstances agglutination may occur and possibly precipitation of the arsphenamin base about to be discussed, but not a coagulation of the globulins or other protein constituents of the plasma.

Coagulation of Blood Proteins by Neoarsphenamin and Other Organic Arsenical Compounds. Solutions of *neoarsphenamin*, *sulpharsphenamin* and *tryparsamid* do not coagulate proteins when added to serum in test tubes even when equal parts of concentrations as high as 10-per cent. isotonic solutions and serum are mixed. In former years, some lots of neoarsphenamin, dissolving with difficulty and yielding cloudy solutions, produced slight precipitation, presumably of the drug, which dissolved immediately upon the addition of a trace of sodium hydroxid, but as manufactured at present, neoarsphenamin produces no visible changes in serum *in vitro* and there are no reasons at all for assuming that coagulation of proteins occurs *in vivo* when this drug is injected intravenously even in concentrated solutions.

Intravascular Precipitation of the Organic Arsenical Compounds in Relation to Toxic Reactions.—In addition to the possibility of intravascular hemolysis, agglutination and coagulation of plasma proteins by solutions of arsphenamin injected intravenously as causes of toxic reactions, the possibility of the formation of precipitates of the drug requires consideration in relation to the mechanism of untoward effects by physical agencies. Danyzs[49] has been especially interested in this subject and has offered an explanation of toxic reactions based upon the hypothesis of intravascular precipitation of arsphenamin and neoarsphenamin worthy of consideration.

Intravascular Precipitation of Arsphenamin.—Danyzs believes that after the intravenous injection of alkaline solutions of arsphenamin that precipitates of the insoluble base may be produced. He states: "A short time after the injection of a disodium solution of arsenobenzol (arsphenamin) this compound loses the sodium which combines with the free carbonic acid forming sodium bicarbonate, leaving the arsenobenzol as an insoluble base. At the same time part of the arsenobenzol which remains as a mono- or disodium salt combines with the calcium phosphates, also producing an insoluble compound. The presence in the blood of free oxygen and sodium chlorid hastens these transformations, while the organic bases contained in the plasma form with the insoluble deposit new soluble compounds."

In other words, Daysz advances the hypothesis that carbon dioxid and the sodium bicarbonate of the blood change arsphenamin into an insoluble base which is carried in the circulation until dissolved by the leucocytes and organic bases of the plasma. The biphosphates of calcium, sodium and magnesium, as well as the chlorids and iron salts of the blood, are alleged to behave similarly. Danysz regards the immediate effects of arsphenamin injections ("réactions du premier degrée") as due not to the toxicity of the drug, but to emboli caused by precipitates resulting from the presence of an excess of certain inorganic ingredients in the blood.

Danysz concludes that the same product can be more or less toxic according as it precipitates more or less rapidly from its solution.

That calcium biphosphate in certain concentrations will precipitate solutions of monosodium arsphenamin in the test-tube, or that unstable or turbid solutions will produce embolism, is easily demonstrable. But to conclude from this experiment that an increase in the calcium biphosphate and other electrolytes in the blood is the cause of the immediate reactions, is a supplementary assertion, which is not, in my opinion, borne out by the facts. Indeed, Jackson and Smith[50] injected intravenously into dogs large doses of various calcium salts and phosphates followed by arsphenamin without in any way increasing the toxicity of the latter. Of interest in this connection is the recent work of Lyman,[51] showing that the calcium content of human blood is quite constant even in pathologic conditions. Jackson and Smith have also studied the effect of an increased carbon dioxid content of the plasma on the toxicity of arsphenamin. They have permitted dogs to rebreathe the expired carbon-dioxid and have observed no influence on the toxicity of the arsphenamin injected, as along as sufficient oxygen was given. The work of Frothingham[52] has, moreover, established the fact that there is no increase in the plasma of bicarbonates in syphilis (and hence no acidosis). An important point not to be overlooked is that the rabbit is unsuitable for the study of this problem, being a herbivorous animal and having a metabolism which is different in many respects from the carnivorous.

From these considerations it is difficult to see, as Rieger[53] has also indicated, how the carbon-dioxid of the blood can abstract sodium from alkaline arsphenamin when it is injected intravenously, even though it may do so in the test tube.

With the assistance of Mr. Joseph L. Gavron, Schamberg, Raiziss, Weiss and I[54] have studied this interesting hypothesis of Danysz with a series of solutions containing the various inorganic and organic constituents in the same proportions as they occur normally in the blood as well as with concentrations of twice that in which they occur in the blood and while it is illogical to infer what may take place *in vivo* on the basis of test-tube experiments, this much is evident: The hypothesis of Danysz is not supported by adequate laboratory experiments either *in vitro* or *in vivo*. We were rather inclined to believe that when arsphenamin is properly nautralized, that is, when disodium and not monosodium arsphenamin is injected, precipitation *in vivo* does not take place.

Jeanselme and Pomaret have claimed that the phenol bodies in arsphenamin may precipitate the plasma proteins, the reaction depending upon the relative acidity of the solution of drug and the speed with which it is injected. They state that the blood of persons who have had "nitrotoid crises" after the injection of arsphenamin has always been found exceptionally acid and that, while a "shock" reaction need not be feared when the bood is highly alkaline, it always occurs when the blood is exceptionally acid.

It would appear, however, from the investigations of others and ourselves mentioned above, that, when the disodium salt of arsphenamin is injected intravenously, precipitates of the drug base are not produced and that such are not to be held responsible for embolism and the production of toxic reactions. Possibly with solutions of the monosodium salt such precipitation may occur and especially if concentrated solutions are injected but if pulmonary embolism and broncho-pneumonia develop under these circumstances agglutination of corpuscles is to be suspected as the cause rather than a coagulation of plasma proteins or precipitation of the drug base. With acid solutions precipitation of the drug is, however, much more likely and may probably add to the production of emboli composed of masses of agglutinated corpuscles, coagulated proteins and the insoluble base of the drug.

Intravascular Precipitation of Neoarsphenamin.—While intravenous injections of neoarsphenamin may produce immediate reactions of flushing, edema of the eyelids, lips or tongue, dyspnea, cough, tremendous fall in blood pressure, etc., and especially the injection of cloudy solutions, there is no evidence that this drug is ever precipitated in the blood and that such physical changes may be responsible for reactions of this kind. Even after the severest of these "nitritoid crises" following the injection of cloudy solutions, the patient does not experience any persistent symptoms and broncho-pneumonia does not appear to follow. Danysz, however, believes that precipitation may occur under exceptional circumstances when the amount of phosphates in the blood is much higher than normal. But he offers no experimental support for this assertion and the established facts in physiologic chemistry speak against it; in the experiments of Schamberg, Raiziss, Weiss and myself referred to above, there were no evidences at all of the precipitation of the drug *in vitro* by any of the organic or inorganic salts of the blood.

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54. Arch. Dermat. and Syph., 1920, 1, 235.

CHAPTER XLVI

CHEMICAL CAUSES OF TOXIC REACTIONS PRODUCED BY ARSPHENAMIN, NEOARSPHENAMIN AND OTHER ORGANIC ARSENICAL COMPOUNDS

Having considered in the preceding chapter the physico-chemical properties of arsphenamin, neoarsphenamin and other organic arsenical compounds in relation to the production of toxic reactions and more especially the changes in the physical (colloidal?) chemistry of the blood which their

administration may produce, we may now discuss the toxic reactions ascribed to direct chemical injury of body cells by the compounds themselves, by the products of their oxidation or reduction as well as by the arsenical and other impurities which they may contain.

These effects may be designated for purposes of simplifying the subject as "chemical injuries," because they are apparently due to effects upon the protoplasm of the fixed cells of certain organs rather than the result of physical colloidal changes in the blood among which may be mentioned those that we may readily evaluate as intravascular hemolysis, agglutination, precipitation and such physical injuries as thrombosis, embolism and infarction. Other types of physico-chemical injury, however, cannot be thoroughly studied by present methods, but while injuries due to these factors are usually referable to dosage and technic of administration, the chemical injuries may be produced by small doses administered by technically correct and acceptable methods. Furthermore, while the physico-chemical injuries in relation to toxic reactions are observed practically only when the compound is injected intravenously, the chemical injuries may be the result of subcutaneous or intramuscular injections as well and produced by numerous small or single large doses.

Chemistry of Arsphenamin, Neoarsphenamin and Other Organic Arsenical Compounds in Relation to Toxic Reactions.—It is practically certain that after the administration of arsphenamin and its substitutes a portion is eliminated unchanged, while the larger part undergoes oxidation or reduction depending upon the trivalent or pentavalent character of the compound. Chemical injury of body cells by unchanged compounds may occur, although elimination of such is ordinarily so rapid that it is reasonable to assume that most damage is due to the products of oxidation or reduction and probably to the ionic arsenic itself; at least, it is customary to estimate the chemical toxicity of these compounds and the results of analyses, in terms of inorganic arsenic since qualitative and quantitative tests for organic arsenic are inaccurate and unacceptable by present methods. It is not possible to say which intermediate compounds between arsphenamin as it is injected and arsenic as it stored and eliminated are of most importance in the production of arsenical injuries, since we know so little regarding the fate of these organic compounds in the body, but it is almost certain that the products of oxidation of which "arsenoxid" is the best known example, are highly toxic and important in these respects. At all events, I believe we may safely conclude that most if not all of the chemical injuries following the technically proper administration of arsphenamin and neoarsphenamin and their substitutes are due to arsenical compounds and these refer not only to the molecules of these substances as injected but more especially to the new compounds produced in the body by processes of oxidation and reduction, as well as to arsenical impurities which commercial samples of the medicaments may contain.

Chemistry of Arsphenamin.—Arsenic therefore, unquestionably constitutes the factor of most importance in relation to the production of toxic reactions by both arsphenamin and neoarsphenamin, but, since it is practically impossible to prepare these compounds upon a large commercial scale in an absolutely pure state, other substances in the nature of impurities are generally present in varying amounts. Indeed, commercial arsphenamin is never a pure substance, but a mixture of dihydroxydiamino-arsenobenzene dihydrochlorid, its oxidation products, inorganic impurities as sulphites or sulphates and sodium chlorid, methyl alcohol, ether, free hydrochloric acid, etc.

Raiziss and Proskouriakoff[1] have found that the arsenic of commercial samples of arsphenamin prepared by different laboratories varied from 30.87 to 32.26 per cent.; according to its chemical formula with either two molecules of water of crystallization or one molecule of methyl alcohol, the compound should contain 31.6 and 31.85 per cent. of arsenic respectively, and the general average for most lots is about 31.6 per cent. as stated by Ehrlich, although the theoretical arsenic content of absolutely pure arsphenamin is 34.2 per cent. According to Kober,[2] methyl alcohol is the chief impurity reponsible for this reduction in the theoretical arsenic and he states it is present in all arsphenamin made according to the original method, but is absent from the arsphenamin prepared from aqueous instead of methyl alcohol solutions. Accordint to Raiziss and Proskouriakoff, a lower arsenic content may be also due to the presence of moisture, ether or other organic solvents the result of insufficient drying as well as to the presence of such inorganic impurities as calcium or magnesium chlorid or salts of heavy metals. Higher arsenic content, on the other hand, they claim may be due not only to the absence of water or methyl alcohol, but also to the presence of such impurities as polyarsenical compounds, arsenophenols or inorganic arsenical compounds.

Raiziss and Proskouriakoff state that, according to the formula of arsphenamin, it should contain 5.89 per cent. of nitrogen; actual analyses of compounds prepared by different laboratories showed from 5.34 to 6.02 per cent. According to these investigators a reduction in the theoretical nitrogen may be due to moisture, methyl alcohol or other organic solvents; also the presence of nitrogen free impurities as arsenophenol or those containing small amounts of nitrogen as dissymmetric compounds, inorganic arsenicals or other inorganic impurities. Higher nitrogen values, on the other hand, may be due to the presence of tetramino-dihydroxy-arsenobenzene tetrahydrochlorid which contains 9.8 per cent. of nitrogen and which may readily appear as an impurity, since the commercial 3-nitro-4-hydroxyphenol-arsenic acid from which arsphenamin is prepared frequently contains the corresponding 3-5-dinitro compound. Since most lots of arsphenamin have a low rather than increased nitrogen content, it is probable that the tetramino compound is only exceptionally present as an impurity, but, according to Raiziss and Proskouriakoff, quantitative estimations of either arsenic or nitrogen alone does not indicate the purity of arsphenamin as well as quantitative estimations of both permitting the expression of a ratio between the two which, for arsphenamin prepared according to the original method, is 31.6 per cent. arsenic and 5.89 per cent. nitrogen or a ratio of 5.37.

When arsphenamin is precipitated from methyl alcohol solution in the final stages of its preparation, it may contain one molecule of this substance, free or loosely combined, along with one molecule of water of crystallization, instead of two molecules of water. At the present time it has not been definitely established, however, whether the formula should be written with two molecules of water or one of methyl alcohol and one of water. Ehrlich and Bertheim[3] themselves found methyl alcohol in some lots and similar findings have been reported by Kober,[4] Rieger,[5] Raiziss, Gavron and Falkov,[6] Binz, Bauer and Hallstein.[7] Kober found that the amount of methyl alcohol, free or loosely combined, is not large, being about 7 per cent. or about 0.042 gm. in a dose of 0.6 gm. Others deny that methyl alcohol is present; Gaebel[8] and Myers,[9] for example, state that it contains two molecules of water and Fargher and Pyman[10] claim that arsphenamin precipitated by the methyl alcohol-ether method contains no methyl alcohol, free or combined. It is possible to prepare arsphenamin without this impurity, but the importance of the subject in relation to toxic reactions has been over emphasized since it is practically certain that the small amounts of methyl alcohol present in well dried preparations produces no harm.

When methyl alcohol is firmly combined with arsphenamin, the resulting methylated compound is apt to be more toxic and of decreased therapeutic activity as shown by Bertheim[11] and Rieger states that commercial arsphenamin may contain an arseniureted methyl compound which decomposes either in the ampule or in solution, with the liberation of arsenous oxid or a cacodyl-like substance, responsible for the vasoparesis of toxic reactions. As arsphenamin is manufactured at present, however, the possibility of the formation of these methylated compounds may be said to be excluded.

It is apparent, therefore, that the chemistry of arsphenamin is exceedingly complex and that commercial lots may contain varying amounts of arsenical or other impurities which may be toxic, although it is exceedingly difficult to properly evaluate these factors in exact terms. All commercial samples probably contain "arsenoxid" in amounts varying from 0.4 to 5 per cent. and this substance is certainly more toxic than arsphenamin itself although not as much so as Ehrlich originally believed. During its manufacture various arsenical intermediates and particularly tetro-amino-dioxy-arsenobenzene may be present in traces in some methods of preparation and some

investigators have reported the occasional presence of inorganic arsenic. The combined sulphur compounds which have been studied by Myers and Dumez[12] are probably of little importance and occur mostly as sulphites or sulphates. Various new sulphur derivatives of arsphenamin have been described which are said to be more toxic and more therapeutically active, but these have not been employed in the treatment of syphilis.

Chemistry of Neoarsphenamin.—The chemistry of neoarsphenamin is probably even more complicated than that of arsphenamin, but the impurities which it contains are not arsenicals and therefore of much lesser toxicity and importance than the impurities of arsphenamin.

The arsenic content usually varies from 18.04 to 19.7 per cent., but this is probably only two thirds of the theoretical arsenic content of pure compounds, the low arsenic content of commercial compounds being due to the presence of various substances as free sodium formaldehyd sulfoxylat, inorganic salts as sodium sulphate and sodium chlorid and other non-arsenical impurities.

Raiziss and Falkov[13] found, when neoarsphenamin was dried at 80°C. in a stream of purified carbon dioxid, that 3.63 to 4.46 per cent. loss of weight occurred which was ascribed to the presence of alcohol or water of crystallization. Qualitative tests did not ordinarily show the presence of any inorganic arsenic and the ratio of arsenic to nitrogen is similar to that found in arsphenamin but subject to greater variation.

The total sulphur varies from 8.25 to 11.13 per cent. due to the presence of formaldehyd sulphoxylate condensed with the amino-group of arsphenamin, free sodium formaldehyd sulphoxylate and occasionally traces of free sodium sulphate or sulphur firmly linked with either the nuclear carbon or arsenic and oxidizable only after the complete breaking down of the molecule. Elvove[13A] has recently described a method for determining total sulphur in neoarsphenamin and sulpharsphenamin which depends on the oxidation of the sample by means of permanganate and hydrochloric acid.

The amount of combined sulphoxylate is usually greater than necesary for one amino-group and less than the theoretical amount for both amino-groups; hence Raiziss and his colleagues have assumed that neoarsphenamin contains a mixture of both the mono- and di-substituted compounds and that variation in the completeness of substitution in the amino-group accounts for differences in toxicity and therapeutic activity of different lots of neoarsphenamin.

All samples contain sodium chlorid, the quantity varying greater from 3.4 to 18.6 per cent., but the presence of this substance is of more importance from the standpoint of influencing the arsenic analyses than from the standpoint of toxicity.

Chemical Constitution of the Organic Arsenical Compounds in Relation to Toxic Relations.—Since the discovery of Ehrlich and Bertheim of the remarkable trypanocidal and spirocheticidal effects of the arseno group (As-As) when attached to the nuclear carbon atom of the molecule of organic arsenical compounds, numerous attempts have been made to introduce such molecular changes as would increase the toxicity of compounds for parasites (parasitropism) while reducing toxicity for the host (organotropism). Despite a great deal of work along these lines, it has not yet been possible to establish definite relations and laws between atomic groupings and biological effects of these compounds. Sufficient data have accumulated, however, to show that with the organic arsenical compounds at least some relation exists, not only between atomic groupings and toxicity for the lower animals as determined by the duration of life after the injection of varying amounts according to body weight, but in relation to gross and microscopical tissue changes as well in the physiologically important organs as the liver, kidneys and cardiovascular system.

In this connection it may be mentioned, for example, that the amino-groups of some of the organic arsenicals may be of as much importance as the arseno-groups in relation to both toxicity and parasiticidal effects, by influencing the amounts of compound or its arsenic anchored or brought into

chemical union with the protoplasm of both parasites and body cells. Raiziss, and Falkov believe that one or both of the amino-groups of neoarsphenamin may be linked with sodium formaldehyd sulphoxylate and that the completeness of the substitution of these groups with this substance accounts for irregularities in toxicity and therapeutic activity. The pioneer work of Ehrlich and his associates had shown quite clearly that, in the preparation of neoarsphenamin, a condensation product of arsphenamin with sodium formaldehyd sulphoxylate, this substitution takes place in one amino group and partially in the second with a consequent reduction in toxicity for both host and parasite but more especially for the former. Studies by Raiziss, Schamberg and myself[14] of additional new derivatives of arsphenamin in which both amino-groups contained fatty acid substitutes, have shown a considerable reduction in the toxicity for both host and parasites (trypanosomes), indicating that both toxicity and therapeutic activity of the organic arsenical compounds is somewhat dependent upon whether the amino-groups are free or substituted.

Of further interest in connection with the importance of the amino-groups is the work of Crabtree,[15] who studied the hemolytic activities of the sodium salts of a number of substituted phenyl arsonic acids and two polyarsenids with the general result that hemolytic activity was observed to increase in direct proportion to the number of amino-groups introduced. As shown by Christiansen,[16] the conditions under which the nitro-group is reduced in the preparation of arsphenamin also influences the toxicity and in a general way slow reduction of this group increases hemolytic activity. According to Crabtree, the introduction of two nitro-groups into dihydroxy-arsenobenzene does not confer hemolytic activity and studies of this kind have indicated quite clearly that substitution of amino-groups may reduce not only the hemolytic properties of the arseno-compounds but possibly their chemical toxicity for the fixed cells of the body as well.

Probably most interest from the clinical standpoint has been placed in the relation of the chemical constitution of organic arsenicals in relation to their toxic action on the optic nerves. There is a general impression that pentavalent compounds are more likely to produce such effects than trivalent compounds and especially atoxyl, arsacetin and tryparsamid, all of which have an amino or a substituted amino group occupying the para position with reference to the arsenic. Young and Loevenhart[17] have recently sought to determine whether the toxic effects of these and other arsenicals for the optic tracts were to be attributed to the pentavalent arsenic, to the presence of the amino group, or to the para position of the amino group. Cats and rabbits were employed in their experiments and the action of the drugs studied by means of opthalmoscopic examinations. The general result of their study was to show that for both trivalent and pentavalent organic arsenicals, those with an amino group or a substituted amino group in the para position were most likely to produce optic nerve changes in these animals. Organic arsenicals with the amino group or substituted amino group in the ortho or meta position to the arsenic, produced no lesions in the rabbit. Arsacetin was more toxic than atoxyl and arseno-phenylglycin least toxic. This led them to conclude that in the group of compounds studied, valence of the arsenic is not the most important factor in the production of the optic lesions, although the valence of the arsenic in certain instances may modify the intensity of action. But the most important factor in the production of optic lesions was found to be the presence of an amino group in the para position to the arsenic.

As will be discussed shortly the tissue changes produced by arsphenamin, neoarsphenamin and other organic arsenical compounds, are probably due in large part to the arsenic, although, as stated by Foulerton,[18] it is impossible to ignore the probability that the tissue toxicity of these arsenobenzenes

may be influenced to some extent by the benzene constituent. Certainly the pathological tissue effects that are produced by arsphenamin and its substitutes, atoxyl, etc., do not bear a definite relation to the amounts of arsenic injected according to body weight. The arsenic of arsphenamin, for example, has a greater effect upon the liver than an equivalent amount of arsenic in neoarsphenamin and the arsenic of the latter is somewhat more toxic for the rat kidney than equivalent amounts of arsenic of the former. In other words, the pathological tissue changes produced by different organic arsenical compounds are known to show wide variation even though animals are injected with equal doses on the basis of arsenic equivalents per gram of body weight.

As previously discussed in Chapter I the chief fundamental principle of chemotherapy is based upon the assumption that changes in the molecule of an organic compound, by adding new side chains, closing or substituting others, may result in increasing its affinity and destructiveness for parasites while decreasing or leaving unchanged its affinity and toxicity for the body cells of the infected host. Unquestionably such possibilities exist, as have been shown by Ehrlich and his co-workers for the organic arsenical compounds, but as recently pointed out by Voegtlin, Dyer and Leonard[19] affinity and toxicity of the organic arsenicals for parasites and body cells may likewise depend, in part at least, upon the presence of some substance in the protoplasm of the parasite or body cell for which the compound has an affinity. For example, these investigators believe that the arsenic of arsphenamin and other organic arsenical compounds in the form of the arsenious oxide (R. As:O) is a specific poison for the sulphhydryl (SH) groups which may occur in the protoplasm of both parasites and body cells, constituting, as it were, the theoretical chemoreceptors of Ehrlich. As discussed in previous chapters, Voegtlin and Homer W. Smith[20] have recorded experimental data to show that, after administration, the organic arsenical compounds must be converted into the trivalent oxids before parasiticidal effects become apparent; arsphenamin and other arsenobenzol derivatives by a process of oxidation and pentavalent arsenicals by a process of reduction. But just how the chemical constitution of the organic arsenical compounds, as, for example, the opening, closing or substitution of the amino-groups mentioned above, affect conversion of the compound into the trivalent oxids in the body and how in turn the affinity of the arsenic of these oxids for the SH groups of the parasites and body cells is involved, has not been determined, but investigations of this kind constitute an important advance in our knowledge of the pharmacodynamics of these compounds and tends to place the subject of chemotherapeutic research upon a much firmer ground than the theory of chemoreceptors of Ehrlich.

Mechanism of Chemical Union of Arsenic with Body Cells.—I have previously discussed in Chapter I the principles underlying the toxicity or organotropism of drugs for body cells, mentioning the theory of chemoreceptors advanced by Ehrlich to explain the union between compound and cells and more particularly the more or less specific tissue affinity of some compounds for certain organs, as well as the recent work of Voegtlin and his associates mentioned above, showing that arsenic is bound to cells by means of the strong chemical affinity between arsenic and the sulphur compounds in the protoplasm of cells. I wish at this time to describe this important work in more detail, as it represents a logical explanation for the mechanism by which arsenic unites with cells, although it does not explain how toxic effects are produced when once arsenic has entered into combination with the protoplasm.

Voegtlin, Dyer and Johnson have drawn attention to the great reactivity of arsenite for hydrogen sulphid and state that "arsenoxid" also reacts with the greatest ease with this sulphur compound as well as with sulphhydryl (R.SH) compounds. It, therefore, seemed quite logical to them to consider the possibility that arsenic might react with a protoplasmic constituent containing sulphur, especially as Heffter[21] in 1908 had demonstrated the fact that the reducing properties of fresh tissues was due to the presence of a "protein-like" substance containing a sulphhydryl (SH) group. Finally, Hopkins[22] isolated from yeast and mammalian tissues a remarkable autoxidizable substance containing a sulphhydryl group designated as "glutathione" which Hopkins and Dixon[23] demonstrated, by means of experiments on surviving frog's and mammalian muscle, was concerned in the processes of oxidation and reduction in living cells.

Voegtlin and his associates state that *Tr. equiperdum* contains an SH group which probably explains the toxic effects of arsenic upon these parasites. Furthermore, they have shown that compounds containing an SH group, like the reduced form of glutathione, sodium thioglycollate, sodium thiosalicylate and glyclycystein, counteract the toxic action of arsenoxid on these trypanosomes, both *in vitro* and in the circulating blood of infected rats, presumably by a union of the arsenic with these compounds by means of their SH group before the arsenic has united with the SH group of the parasites. In addition, these investigators claim that the administration of glutathione, sodium thioglycollate and the other SH compounds previously mentioned, greatly reduced the toxicity of arsenoxid for the white rat, delaying both the onset of toxic symptoms and the time of death, presumably by uniting with and detoxifying a portion of the arsenic before the latter had united with the body cells. It is of particular interest to note that in their experiments sodium thiosulphate and sulphite were without effect, although the former substance has been advocated for the treatment of arsphenamin jaundice and dermatitis. The coorresponding disulphids (RR.S-SS.R) of the above mentioned SH compounds were much less effective or practically without effect and amino-acids containing no SH group as well as glucose, lecithin, and inorganic salts, were without detoxifying activity.

On the basis of these experiments, Voegtlin and his associates have advanced the theory that arsenic in the form of the trivalent oxids (R.As:O) is a specific poison for the sulphhydryl (SH) group of glutathione and possibly other SH compounds which may occur in the protoplasm of living cells. They have shown that SH compounds injected intravenously are partly oxidized and may protect trypanosomes against the toxic action of arsenic or more importantly, diffuse in part into the tissues and protect the cells against arsenic. Voegtlin, Dyer and Leonard[24] have found that an intravenous injection of reduced glutathione inhibits the toxic action of arsenic in rats. It was also found that the toxicity of arsenic was reduced by feeding rats with a mixture of glutaminic acid and cystine, indirect evidence being obtained on the biochemical synthesis of glutathione from the glutaminic acid and cystine contained in the diet. Cystine however, was found less effective than glutathione as an antidote against arsenic poisoning but the data indicate in the opinion of Voegtlin and his colleagues, that the SH group of glutathione may be regarded as the so-called "arsenic receptor" of mammalian protoplasm.

Whether or not the administration of SH compounds as sodium thioglycollate and sodium thiosalicylate results in counteracting the toxic effects of arsenic when the latter has already united with cells and produced symptoms of arsenical poisoning, is not stated in their report, but the subject is of much practical importance in relation to the treatment of the toxic reactions due to arsenic following the injection of arsphenamin, neoarsphenamin and their substitutes.

Specific Tissue Affinities of the Organic Arsenical Compounds.—If the arsenic of such medical compounds as atoxyl, arsphenamin and its substitutes, etc., enters into chemical combination with the protoplasm of living cells by means of the sulphur (SH) compounds of the latter, how may one explain the greater affinity shown by some arsenical compounds for certain organs? And there can be no doubt that such selective affinities exist among the organic arsenical compounds which appear to bear a relation to chemical constitution, as shown by the gross and microscopical tissue studies of Pearce and Brown with such compounds as arsenious acid, atoxyl, arsacetin, arsenophenylglycin, arsphenamin, neoarsphenamin, etc. Furthermore, as will be later discussed, the arsenic of neoarsphenamin appears to have a greater effect upon the kidneys of the lower animals than equal amounts of arsenic in arsphenamin per kilogram of body weight. Of course, it is to be

expected that such organs as the kidneys and liver may show the maximum degrees of injury by reason of their enunctory functions or, as in the case of the liver, by reason of storage of arsenic in addition, but my remarks are concerned more with the question of why one type of arsenical will produce predominantly vascular injuries of organs while with another the changes are mainly parenchymatous, etc., showing a difference in affinity for different cells in the same organ. It may be that the atomic groupings of the compound determine primarily its affinity for certain organs or cells, although if Voegtlin is correct that the organic arsenicals are oxidized or reduced to trivalent oxids before toxic effects are produced and finally to inert pentavalent compounds, it is difficult to understand the mechanism of the apparent selective affinities of some arsenical compounds for certain organs. Possibly the distribution of sulphhydryl compounds in the cells of different organs may be partly responsible as affecting chemical affinities, but the subject is one requiring further investigation for accurate information.

Mechanism of the Toxic Action of Arsenic.—Before, however, considering the pathological tissue changes and toxic symptoms that may be ascribed to the chemical combination of arsenic with cells following the administration of arsphenamin and its substitutes in the treatment of syphilis, it may be well to briefly discuss prevailing views upon the pharmacodynamics of arsenic, that is, the mechanism of the action of arsenic upon the protoplasm of living cells.

Largely on the basis of the investigations of Binz and Schultz,[24a] it is commonly stated that the toxic effects of arsenic upon cells is due to interference with protoplasmic oxidation. These investigators advanced the theory that arsenious acid is oxidized to arsenic acid in the living cells, and the latter again reduced to arsenious acid, the alternate oxidation and reduction withdrawing and supplying oxygen and in this way exerting injurious effects. If this were true both forms of arsenic should possess the same toxicity, which, as shown by subsequent investigators, is not the case for the higher mammals; as stated by Cushny, "while arsenic acid appears to be reduced and arsenious acid oxidized in the tissues, these processes are probably only gradual. Otherwise it would be difficult to explain how arsenious acid is so much more poisonous than arsenic acid; for if the latter were readily reduced to arsenious acid, it would be equally toxic." Sollmann also raises objection to this oxidation and reduction theory and states that it is not tenable. In other words, we do not yet know how arsenic acts in the living cells and particularly how it produces toxic or abnormal changes. We know considerable concerning the histological changes that may be produced and likewise of the resulting gross lesions and consequent symptoms, but of what goes on after the arsenic has entered into combination with the protoplasm we are yet in ignorance. Possibly the recent work of Voegtlin and his associates just described, showing that arsenic may enter into combination with cells by means of the sulphur compounds in the protoplasm of the latter, may open a new channel for investigation and show that disturbances in the sulphur metabolism, rather than interference with protoplasmic oxidation, is of most importance in this connection.

Toxicity of Arsphenamin and Its Substitutes for the Lower Animals in Relation to Toxicity for Human Beings.—As stated in preceding chapters toxicity tests with the lower animals have only a broad and general relation to the subject of toxic reactions in human beings. The Hygienic Laboratory requires that arsphenamin (disodium salt) shall be borne by rats for at least forty-eight hours in dose of 0.120 gm. per kilo; this is about 12 times more per body weight than ordinarily administered to human beings at one time (0.6

gm. per 60 kilograms or 0.010 gm. per kilo). The requirements for neoarsphenamin are that rats shall bear at least 0.240 gm. per kilo for seven days which is about 16 times more per body weight than ordinarily given a human adult in one dose (0.9 gm. per 60 kilograms or 0.015 gm. per kilo); sulpharsphenamin must be borne in dose of 0.300 gm. per kilo by intravenous injection and sodium arsphenamin in dose of at least 0.180 gm. per kilo.

These requirements would appear to be sufficiently safe; probably little is to be gained by raising them since compounds fulfilling these conditions may still produce toxic reactions in human beings due to a wide variety of factors connected with the patient or method of administration which cannot be checked or evaluated by animal tests. In other words, an animal test which requires that a medicament shall be borne in a dose at least 10 times larger than the amount given at one time to human beings according to body weight, would appear sufficient for determining whether the compound is sufficiently safe and acceptable from the standpoint of what may be termed as "gross toxicity;" it has occurred time and again in my experience that compounds and especially arsphenamin, fulfilling these requirements may still produce toxic reactions in human beings and I doubt if these can be avoided by raising the requirements of the rat toxicity test, although it is plain that an arsphenamin passing at 0.150 gm. per kilo is to be preferred to one just passing at 0.120 per kilo, *provided the therapeutic activity is at least the same for both.* Likewise, a neoarsphenamin passing at 0.400 gm. per kilo is to be preferred to one just passing at 0.240 gm. per kilo, provided there is no loss in spirocheticidal power; I know, however, *that not infrequently brands of neoarsphenamin lauded by their manufacturers on the basis of low toxicity are likewise of reduced therapeutic activity. In other words, technical procedures tending to reduce toxicity of neoarsphenamin and its substitutes for the host may also result in a reduction of toxicity for Spirochæta pallida and Tr. equiperdum, and I repeat as my opinion that manufacturers may do well to direct more effort toward increasing the spirocheticidal activity of their compounds while maintaining the toxicity requirements of the Hygienic Laboratory.*

The toxicity values expressed above were for arsphenamin and its substitutes as these are commercially available; they do not refer to the toxicity of absolutely pure compounds as such are not ordinarily available. The toxicity is due, therefore, to the presence of some impurities as well and it may now be profitable to enquire more closely into the toxicity of some of these substances.

Toxicity of Impurities in Arsphenamin and Neoarsphenamin for the Lower Animals. *Toxicity of Arsenoxid and Other Arsenical Impurities.*—In 1912, Ehrlich and Bertheim[25] stated that "the hydrochloride of di-amino-dihydroxy-arseno-benzene shares with other arsenic compounds the peculiarity that it is easily oxidizable. When it is exposed to the air, it will quickly form amino-hydroxy-phenylarsenoxide; however, the formation of an arsenoxide compound takes place immediately, if the experiment is carried on in the usual salt bottle. This fact, is therefore, of the greatest significance in the practical application of the substance as a curative agent, because the arsenoxid is about 20 times as toxic as the pure hydrochlorid of an arseno-compound."

Since then *arsenoxid* (3-amino-4-hydroxyphenyl-arsenoxid) has been most prominently discussed in the literature as a cause for toxic reactions and probably constitutes an important toxic agent, although it is not as toxic for the lower animals as Ehrlich originally surmised. Arsenoxid may be produced by the oxidation of the arsphenamin powder in the ampule and especially if there is a crack in the glass; or it may be produced during the prepara-

tion of the solution for injection and especially by excessive shaking of the alkalinized solution. Myers[26] states that all commercial samples contain from 0.4 to 5 per cent. arsenoxid. The purer forms of arsphenamin are not freely soluble in cold water as previously stated and require the use of hot water for effecting quick solutions; arsenoxid, however, is not produced in appreciable amounts by dissolving arsphenamin in hot water and shaking arsphenamin in hot or cold water has very little effect, although excessive shaking *after* the addition of sodium hydroxid results in increasing the toxicity presumably in part at least by the formation of arsenoxid. However, these are matters largely concerning the toxicity of these compounds in relation to the technic of administration and the subject is discussed with more detail in Chapter XLVIII.

For the white rat the maximum tolerated dose of 2-per cent. solutions of the hydrochlorid of arsenoxid is from 0.020 to 0.030 gm. per kilo of weight. It is, therefore, about 5 times more toxic than disodium arsphenamin for rats. According to Hata,[27] the maximum tolerated dose for mice by subcutaneous injection is 0.0165 gm. per kilo, for rats by intravenous injection 0.035 gm. per kilo and for rabbits by intravenous injection about 0.015 gm. per kilo. According to Hunt,[28] the intravenous injection of this substance produces characteristic symptoms in the rat and when 10 per cent. of arsphenamin in a sample was replaced by an equal weight of the oxid and injected into rats in doses of from 0.100 to 0.120 gm. per kilogram, the symptoms were characteristic of the oxid, and entirely unlike those caused by any other compounds tested by Hunt, with the exception of the analogous sulphid. The further oxidation of "arsenoxid" results in the production of m-amino-p-hydroxyphenyl arsonic acid, which is much less toxic than arsphenamin.

Regarding the toxicity of other arsenical impurities in arsphenamin and neoarsphenamin for the lower animals we have but little information. Inorganic arsenic is occasionally found in arsphenamin but in such small amounts as to be almost negligible although the arsenites are very toxic, the maximum tolerated dose for the rat being about 0.003 gm. per kilo. Traces of the tetra-amino-compound may likewise be found occasionally in arsphenamin manufactured by some methods, but this impurity is usually absent in present day products.

Toxicity of Methyl Alcohol and Inorganic Impurities.—From the standpoint of toxicity, the importance of methyl alcohol has been over emphasized. In the small amounts that may be present, it cannot be held responsible for toxic reactions and is of chief interest in relation to the chemistry of arsphenamin and especially as an explanation in part for the presence of less than the theoretical amount of arsenic, as discussed above. I have found that white rats can bear without apparent injury intravenous injections of methyl alcohol in doses as high as 1 c.c. of 10-per cent. solutions per kilo, which is much larger than the traces to be found in arsphenamin.

The hydrochloric acid of arsphenamin dissolved in water without neutralization which may amount to about 0.2 per cent. of the free acid in addition to that which is combined with the two amino-groups, unquestionably is a toxic factor of great importance, but more especially in relation to injuries due to changes in the physical colloidal chemistry of the blood as discussed in the preceding chapter; furthermore, since acid solutions of arsphenamin are not injected intravenously except by accident, I shall discuss the subject with more detail in Chapter LI.

Whether or not the sulphites or sulphates and small amounts of sodium chlorid present are capable of producing toxic reactions cannot be stated; for the rat the toxicity of these is so slight symptoms are not produced by the injection of the amounts found in 0.100 to 0.200 gm. of arsphenamin and neoarsphenamin per kilogram of weight, although it is possible that the sodium chlorid may produce fever and other slight symptoms in human beings.

Toxicity of Impurities in Arsphenamin and Neoarsphenamin for Human Beings.—It is extremely difficult however, to evaluate the importance of the arsenical and non-arsenical impurities in arsphenamin, neoarsphenamin and their substitutes in relation to the toxic reactions observed in human beings under treatment with these compounds.

Undoubtedly, the products of oxidation which may be present in arsphenamin, neoarsphenamin and their substitutes, as dispensed commercially or which may be produced during the process of preparing the solutions for injection and of which arsenoxid is the best known example, are of most importance; at least, these "oxids" have commanded most attention from the standpoint of toxicity. As stated above, direct toxicity tests with prepared arsenoxid have shown that it is at least 5 times more toxic than disodium arsphenamin and if arsphenamin is improperly dispensed so that oxygen remains in contact with the powder or if the ampule cracks permitting an access of oxygen, it is possible that oxidation will occur with an increase of toxicity. It is commonly stated that oxidation also occurs during the preparation of the solutions and especially if these are excessively shaken; as discussed in Chapter LVIII, the importance of this factor is over emphasized, because arsenoxid production occurs very slowly in solutions of arsphenamin in water (acid solutions) but more quickly in alkalinized solutions; it is not necessary, however, to give the latter more than one or two shakes before filtering and injecting the solution.

It is possible but not probable that traces of intermediate organic arsenical compounds or of inorganic arsenic in arsphenamin, neoarsphenamin and other medicinal organic arsenicals may produce toxic symptoms, but the evidence is not clear and chemical analyses for such have generally yielded negative results.

It is practically certain that the traces of methyl alcohol present in some lots of arsphenamin are of no importance in relation to toxic reactions. The sulphur compounds, and especially those present in neoarsphenamin, may be of more importance in this connection, but animal tests have shown these to be so slightly toxic that they have commanded little or no attention. Other inorganic constituents and especially sodium chlorid may be partly responsible for such mild reactionary symptoms as fever, but the evidence is not clear.

As stated above, the oxidation products, of which arsenoxid is the best known example, have been regarded as most important in relation to toxic reactions. But both Myers and Hunt state that they have not found any commercial preparations of arsphenamin containing sufficient of this substance to account for toxicity. In other words, they believe that toxicity is due primarily to factors other than arsenoxid, inorganic arsenic and intermediate arsenical compounds when arsphenamin is properly prepared and administered. I believe this is true insofar as the immediate toxic reactions are concerned; the late toxic effects as dermatitis, hepatitis with jaundice, nephritis, etc., are, however, to be ascribed to arsenic, but primarily to the arsenic of the compounds themselves rather than to arsenical impurities.

To what then may the early toxic reactions be ascribed? There can be no doubt that some lots of arsphenamin and neoarsphenamin produce such a high incidence of toxic reactions that their administration must be stopped. In 1917, Schamberg, Raiziss and myself advanced the theory that toxic reactions may be due to an impurity in some lots of arsphenamin and neoarsphenamin designated for convenience as "*substance X*." But all attempts to identify this hypothetical substance have failed. Time and again toxicity tests with rats have failed to show an increased toxicity of compounds

that produced a high incidence of toxic reactions in human subjects and chemical analyses for arsenic and arsenical impurities have failed to disclose a possible explanation.

I now believe that the toxicity of these compounds is due to physical factors and that "substance X" is in fact to be ascribed to changes in hydrogen ion concentration of the solutions, viscosity of the solutions and other physical factors modifying the physical colloidal chemistry of solutions of these compounds as administered or subsequently the colloidal chemistry of the blood. It is well known, for example, that cloudy solutions of neoarsphenamin are much more likely to produce reactions than clear solutions and the whole subject has been discussed with more detail in the preceding chapter. It is not uncommonly observed that if several individuals given equal doses of the same arsphenamin or neoarsphenamin that some develop toxic reactions while others do not; under these conditions an impurity should be toxic for all. Such results, however, are not to be ascribed to an impurity but rather to physical factors involving the colloidal chemistry of the solution of medicament or individual changes in the physical chemistry of the blood after injection. Furthermore, toxic reactions by individuals to whom the drugs are given in a technically perfect manner may be due to hypersensitiveness or to pathological tissue changes peculiar for the individual and not to be ascribed to either physical or chemical toxicity on the part of the compound administered.

Insofar as immediate or early toxic reactions are concerned, I believe, therefore, that these are to be ascribed more to physical colloidal factors than to arsenical or other impurities. On the other hand, some immediate and early reactions are due in my opinion to congenital or acquired hypersensitiveness to arsenic and under these circumstances, early reactions may be caused by the arsenic of the compound itself without bringing into consideration arsenical impurities. As stated above, the late toxic reactions as dermatitis, hepatitis, nephritis, etc., are doubtless due in large part to the pathological changes produced by arsenic, but here again these effects may be due to the arsenic of the compounds rather than to arsenical impurities.

Tissue Changes Produced by Arsphenamin, Neoarsphenamin and Organic Arsenical Compounds in Relation to Toxic Reactions.—And now we may with much profit briefly review the subject of the tissue changes that may be produced by the arsenical compounds employed in the treatment of syphilis and other diseases in relation to the production of toxic reactions. The possibility of the production of these changes is not always kept as much in mind in chemotherapeutic research and even in the practical treatment of disease, as their importance warrants. It is true that histological changes may exist at least for a time, without the production of manifest symptoms and more especially, that symptoms may be apparently present for a time without discernible tissue changes, but to judge the toxicity of a new compound for human beings solely on the basis of whether or not clinically toxic reactions and symptoms are produced or on the basis of symptoms and duration of life in the case of the lower animals, is not sufficient and pathological tissue changes may be in some cases the basis for toxic reactions and untoward or dangerous effects.

Of course, most interest is to be placed upon the possible pathological changes that may be produced by those organic arsenicals employed at the present time for the treatment of disease as atoxyl, arsphenamin and neoarsphenamin and their substitutes; likewise most interest is to be placed on the possible injurious effects of these compounds upon the physiologically important organs as the cardiovascular system, brain and meninges, liver,

kidneys and adrenals and especially the effects that may be produced by multiple small therapeutic sized doses rather than single or multiple maximum tolerated doses. The latter are required in a study of experimentally produced lesions in order to intensify the pathological changes, but due care must be exercised against drawing conclusions based exclusively upon such results.

Practical Value of Chemopathological Studies with Compounds of Arsenic.— It may be stated, therefore, that studies of the gross and microscopical tissue changes produced by arsenical compounds are of practical value in several directions, namely: (1) as a means for studying the relationship between chemical constitution and organotropism (toxicity); (2) for determining the special affinity of a compound for certain tissues and organs and (3) in relation to the mechanism of production of toxic reactions and untoward results. All are of special interest to the experimental chemotherapeutist and particularly the first mentioned, while the two latter are of special interest in relation to the practical treatment of syphilis and other diseases. In other words, the physician should always keep carefully in mind that the condition of the patient as well as the disease require consideration and that repeated doses of a medicament as arsphenamin and neoarsphenamin, and more especially mercury, may produce profound tissue changes and in some cases render the treatment more dangerous than the disease. This is not likely to be the case in acute early syphilis with otherwise healthy internal organs, but in chronic syphilis, persistent and long continued efforts to eliminate the infection may result in damaging already diseased and impaired organs. In other words, a good working knowledge of the possible harmful tissue changes that may be produced by the chosen medicament will go far toward a wise selection of doses and spacing of injections, as well as engender a policy of watchful waiting for the earliest evidences of nephritis or other tissue injuries and the employment of rational and efficient means for their prevention and treatment.

Influences of Chemopathological Tissue Changes upon the Course of Syphilis. Aside from the question of the pathological tissue changes that may result from the chemical toxicity of the organic arsenical compounds employed in the treatment of syphilis and other diseases, the question naturally arises, may not the production of such injuries constitute foci of lowered tissue resistance to *Spirochæta pallida* or other parasites and thereby favor dissemination of the infection? This point has been raised in connection with the pathogenesis of neurosyphilis on the basis that too vigorous treatment with arsphenamin or its substitutes may indeed produce vascular injuries and favor infection of these tissues and is not lightly to be dismissed when one recollects in this connection that arsenic is especially apt to produce vascular injuries which may readily enough open up a way for infection of the perivascular spaces if spirochetes happen to be in the blood. It is my opinion that temporary periods of spirochetemia may occur at any time during the course of syphilis, as based upon those instances of successful inoculation of the testicles of rabbits with blood in the different stages, and it may be that in these periods the spirochetes in the blood may find conditions for infection particularly good in tissues primarily injured by arsenic. One may naturally assume that the cells of such areas contain arsenic and that the reverse would be true to the effect that spirochetes finding lodgement would undergo destruction, but we cannot be sure that the arsenic present in such form is spirocheticidal and available data indicates indeed that it is not.

Probably most physicians with experience in the treatment of syphilis have observed in some chronic cases an exacerbation of the lesions and cer-

tain symptoms shortly after the institution of specific treatment well known as the Jarisch-Herxheimer reaction; these reactions, however, are to be ascribed primarily to the stimulation of the spirochetes in pre-existing lesions and, while they may be followed secondarily by a further spread of the disease, one cannot say that either the primary or secondary effects are due to a lowering of resistance by pathological tissue changes endengered by the drug and especially in the case of arsphenamin or neoarsphenamin properly administered in the usual doses.

In other words, it is possible but not at all probable, that arsphenamin and neoarsphenamin may actually favor the dissemination of syphilis or progression of lesions by producing areas of lowered resistance in the nature of pathological tissue changes, but there is no proof of such and clinical experience is against the acceptance of a hypothesis of this kind.

Influence of Pathological States Due to Disease or Produced by Drugs upon the Chemopathology of Arsphenamin and Neoarsphenamin.—However, the converse of this may be true—that is, pathological changes in the kidneys or liver, engendered by syphilis, some other disease or by the previous administration of mercury or bismuth, may influence the production of further tissue changes and consequent toxic reactions by arsphenamin and its substitutes by apparently interferring with the elimination of these arsenicals

Pearce and Brown have stated that it is doubtful whethere there is as yet a single compound of arsenic possessing curative value in experimental trypanosomiasis that produces neither toxic symptoms nor organic tissue changes. I believe that this is true when very large doses are administered, but it is well established that such experimental infections as acute early trypanosomiasis and syphilis may be cured by doses of arsphenamin and neoarsphenamin producing neither toxic symptoms nor tissue changes; I believe the same applies in the treatment of the majority of cases of acute early syphilis of otherwise healthy human beings, but when treatment is complicated by the existence of pathological tissue changes produced by chronic syphilis, some other infection or drugs and especially of the liver, kidneys and cardiovascular system, the question of additional injury due to treatment with arsphenamin or its substitutes becomes a matter of primary importance, and especially if elimination of the compounds through the kidneys and liver is reduced.

Wechselmann[29] has drawn particular attention to the possibility of mercury so injurying the kidneys as to result in the prolonged retention of the oxidation products of arsphenamin and thereby favoring the production of acute toxic reactions and especially a type of acute meningeal congestion with edema designated in Europe as "hemorrhagic encephalitis." In an analysis of fatalities from salvarsan up to 1914, Wechselmann concluded that when these could be ascribed to the drug properly administered, that in almost all there was a history of preceding mercurial treatment, and he accordingly issued a warning for extra caution in the treatment of syphilis with salvarsan following the administration of mercury on the basis that encephalitis was due to insufficiency of the kidneys rather than to hypersensitiveness of the brain and meninges.

One may not agree with all of these statements, but it cannot be denied that any pathological condition interfering with the elimination of the oxidized products of arsphenamin and other arsenicals may be expected to increase the chances for the production of pathological tissue changes and doubtless an organ, as the liver, previously damaged by syphilis, is more susceptible to further damage by arsphenamin since it is well established that arsenic is both eliminated and stored in this organ. In brief, the question of

pathological tissue changes that may be produced by arsphenamin or neoarsphenamin is to be considered quite a different problem in the case of chronic syphilis than in a normal healthy lower animal, since in the former predisposing tissue changes due to the disease or previous mercurial and bismuth medication are apt to be present.

Difficulties and Methods in Determining the Chemopathological Tissue Changes Produced by Arsphenamin and Its Substitutes; Influence of Fasting and Diet.—It is by no means easy, therefore, always to determine accurately in cases succumbing after the administration of arsphenamin or one of its substitutes whether death was due primarily to the drug. Of course, in those unfortunate fatalities following the intravenous injection of concentrated solutions of arsphenamin hydrochlorid (acid solutions) little or no difficulty is encountered in determining the cause of death, but when the drug has been properly administered greater difficulties are encountered in differentiating those tissue changes that may be due to syphilis or some other disease or drug (especially mercury) from those produced by arsphenamin or its substitutes. In the tissues of cases which I have had the opportunity of examining both of these factors required consideration and in none could it be stated with conviction that death was due primarily and solely to the drug excepting in one case to whom a physician had administered 0.6 gm. arsphenamin dissolved in 18 c.c. of water without neutralization in mistake for neoarsphenamin.

As stated by Wechselmann, deaths following subcutaneous injections of arsphenamin can hardly be ascribed to the drug as the patients have usually suffered from the severest syphilitic involvements of the internal organs to whom salvarsan was administered as a last resort. The same may be said of those fatalities following intramuscular injections. Fatalities after intravenous injections of arsphenamin have been more common and a few have occurred following injections of neoarsphenamin, but it may be said that many of these, probably the majority, were due either to errors in the technic of administration and especially of arsphenamin, or to severe syphilitic, malarial or other degenerative lesions. One is impressed upon reviewing necropsy records with the large number of instances in which active tuberculosis, widespread syphilitic aortitis and generalized arteriosclerosis, aneurysms, chronic leptomeningitis (especially in Europe in cases of chronic alcoholism), etc., have been found. Except in those cases dying of acute yellow atrophy of the liver in which arsenical poisoning is always to be first suspected it is indeed difficult to decide what relation arsphenamin or its substitutes bore to the cause of death and especially in those cases of chronic alcoholism, severe syphilitic or other infections and chronic illnesses succumbing during treatment with these compounds.

For these reasons the pathological tissue changes that may be produced by arsphenamin and its substitutes must be studied primarily by administering the compounds to the lower animals and especially the dog, rabbit, guinea-pig and rat in order to obtain precise information in this subject. By arranging the doses according to body weight, one may duplicate conditions pertaining to human beings; but here again it is necessary to bear in mind that the kidneys of healthy lower animals, and especially of the dog and rabbit, may show chronic changes, and the brains of monkeys and some rabbits may show varying degrees of perivascular lymphocytic infiltrations, which may be readily enough ascribed to the effects of the drug under study. In my own work I have found healthy white rats especially serviceable for studying the tissue changes produced by arsphenamin and its substitutes, since the animals may be maintained on a mixed diet and repeatedly injected. In my expe-

rience the tissues of normal healthy adult rats on a mixed diet do not show spontaneous chronic lesions as mentioned above in the kidneys of rabbits and these animals are to be especially recommended for histo-pathological studies in chemotherapeutic investigations. In this connection it is worthy of note that Hooper, Kolls and Wright,[30] reviewing the literature upon the influence of fasting and various diets on the toxicity of various drugs for the lower animals and studying the question with special reference to the toxicity of arsphenamin and neoarsphenamin for white rats, observed that the tissue changes and especially nephritis, were most severe in starved animals and those fed sugar alone preceding the injections; a diet of white bread, rolled oats, and whole milk was observed to greatly increase the resistance of these animals and to exert a marked protective action against the kidney injury.

Special care must be exercised when rabbits are employed for studies of this kind. As pointed out by Ophuls, Hooper and Myers and others a large percentage of rabbits have spontaneous kidney lesions and more or less cirrhosis of the liver with round-cell infiltration as a rule rather than the exception. They have further encountered areas of round-cell infiltration in the heart muscle and brain, areas of bronchopneumonia and arteriosclerosis. It is evident therefore that all histopathologic deductions on rabbits should be made with a full knowledge of the occurrence of the spontaneous lesions. This would especially apply to experiments with the arsenic and mercury or bismuth compounds where the drugs are known to produce kidney or liver damage when administered in toxic doses and I shall have occassion to refer to this subject again in relation to the interpretation of histopathological changes in rabbits.

Varying Pathological Tissue Changes Produced by Different Arsenical Compounds.—It cannot be said that the different organic and inorganic compounds of arsenic produce the same kind of tissue injuries; there is no one "type." Rather, as stated by Wechselmann in reference to the kidney lesions, the effects produced by arsphenamin, atoxyl and arsacetin vary considerably and the studies of Pearce and Brown[31] with arsenious acid, salvarsan, neosalvarsan, galyl, arsacetin, arsenophenylglycin and atoyxl administered to dogs, have amply shown that all arsenical compounds do not produce the same type of renal injury. In general, they observed two broad groups of lesions, the red and the pale kidney, with a variety of subdivisions of each group depending upon modifications in the chemical constitution of the compound, dosage, and length of survival of the animal. Histological studies have shown them[32] that, while some arsenicals produce changes in which vascular injury predominates, others produce an equally dominant tubular injury, each compound producing a lesion-comp ex of the kidney that was relatively characteristic for that compound. Pearce and Brown[33] also observed that in guinea-pigs the tissue changes in the adrenal glands of congestion, hemorrhage, disturbances in the lipoid content, cellular degenerations and necroses, and reduction in the chromaffin content varied in character and severity with these different arsenicals according to their chemical constitution and Brown[34] has described varying lesions in other organs as the heart, lungs and liver as well according to the kind of compound administered.

For details regarding the gross and microscopical changes in the kidneys and adrenals of the lower animals produced by arsenious acid, arsacetin, arsenophenylglycin and atoxyl, I would refer the reader to the papers by Pearce and Brown; since only arsphenamin and its substitutes, tryparsamid and atoxyl, are employed at present in the treatment of syphilis, frambesia, trypanosomiasis, etc., I shall confine my remarks entirely to the tissue changes which these compounds have produced in the lower animals and in the case of arsphenamin, to the tissue changes in fatal dases of poisoning of human beings whose tissues I have had the opporunity of studying histologically. Lucke and I[35] have been particularly interested in the histological

changes produced in rabbits and rats by arsphenamin and neoarsphenamin and since a detailed description of our observations along with photomicrographs of tissue changes are to be found in our papers, I shall review the subject here very briefly.

Pathological Tissue Changes Produced by Arsphenamin.—A clear distinction is usually to be made between the pathological tissue changes produced by the intravenous injection of solutions of arsphenamin hydrochlorid in water (acid solutions) and solutions which have been properly neutralized before injection. When the former are injected intravenously, severe reactions are usually produced immediately in both human subjects and the lower animals which may terminate fatally. If death occurs at once or within a few hours, the chief lesions are widespread and intense hyperemia of the brain and meninges, lungs, liver, kidneys and other organs with extensive thrombosis of agglutinated erythrocytes and plasma precipitates. In some organs small capillary hemorrhages may be found, but degenerative lesions are not produced unless survival is for a day or more, although in rabbits Lucke and I observed early parenchymatous changes in the liver and kidneys of the lower animals as early as five hours after injection of acid solutions of arsphenamin. Under these circumstances immediate death is due, therefore, primarily to acute vascular and intravascular changes of which congestion, thrombosis and embolism (especially of the lungs) are the prominent and sometimes sole lesions; in human subjects succumbing within twenty-four hours after the accidental administration of these acid solutions, advanced degenerative lesions in the liver, kidneys and other organs are to be ascribed more to previous medication or infection since the time is too short under these circumstances for the production of severe arsenical degenerations.

The pathological tissue changes produced by *neutralized and alkalinized solutions of arsphenamin (disodium salt)*, as administered by intravenous injection, vary considerably according to dosage. Insofar as the lower animals are concerned the acute lesions can be produced only by massive doses, as 0.100 gm. per kilo or more; some human beings have succumbed after the injection of 0.010 gm. per kilo, but in these cases a hypersensitiveness to arsenic was probably present which is not encountered among the lower animals. The majority of fatalitites among human subjects due to arsphenamin properly administered have occurred, however, after several doses had been given, death being due to chronic rather than acute poisoning.

A great deal of interest is to be placed upon the tissue changes that may occur in the *brain and meninges*, since in Europe salvarsan fatalities have been frequently ascribed to acute congestion with edema of the meninges variously designated as "encephalitis hemorrhagica," "serous apoplexy," etc. In the United States fatalities have not been ascribed to these lesions, but rather to liver and kidney degenerations and the question has been sometimes raised whether or not the original salvarsan of German manufacture was more prone to produce these lesions than the products manufactured in this country.

As far as I am able to determine, the changes in the meninges and brains of rats receiving 0.100 gm. per kilo and in rabbits receiving 0.080 gm. per kilo were identical with both the original salvarsan as prepared in 1914 and 1915 and the products of more recent manufacture in this country. In animals succumbing within two to five days after these doses Lucke and I found no striking or constant changes and after the injection of 8 to 10 doses of disodium arsphenamin in amounts of 0.010 gm. per kilo which corresponds to 0.6 gm. for about 132 pounds, only inconspicuous changes were found like a slight degree of hyperemia of the meninges with no cellular

infiltrations. It is apparent, therefore, that the vessels of the meninges and to a lesser extent of the brain may show the same vasomotor changes of acute congestion as is found widespread in other organs although these changes are not conspicuous with doses corresponding to those administered human beings. As I shall discuss later, there is considerable evidence to show that hypersensitiveness to arsenic, either natural or acquired, undoubtedly occurs in some human beings and may contribute in no small part to the toxic reactions. As is true of all allergic phenomena vasomotor paralysis with acute hyperemia and serous exudation constitutes the chief lesions and it may be that in those salvarsan fatalities ascribed to "hemorrhagic encephalitis," "serous apoplexy," etc., the effects were due to this allergic hyperemia and exudation in addition to the well-known effects of arsenic in producing vasomotor disturbances with acute hyperemia.

After single large doses, Lucke and I observed no constant or striking changes in the *heart* although after 10 or more small doses the hearts of some rats showed irregularly distributed focal areas of degeneration situated sometimes near the endocardium, sometimes beneath the epicardium or deep in the myocardium, with lymphocytic infiltrations which were of special interest in view of the fact that such changes found in the heart muscle of human cases of syphilis may be regarded as foci of spirochetic infection rather than an effect of the drug.

As would be expected, vascular lesions are particularly prominent in the *lungs* and any intravascular blood changes as agglutination or plasma protein precipitation following intravenous injections are particularly apt to show pulmonary embolism. It is no wonder, therefore, that pulmonary symptoms and lesions occupy a prominent place in the toxic reactions of arsphenamin and even after acute symptoms have subsided, bronchopneumonia may follow. After single large doses, Lucke and I observed that vascular changes were prominent and when examined within two days after injection of arsphenamin, marked congestion and small thrombi of conglutinated erythrocytes were seen associated with scattered areas of edema and extravasation of erythrocytes into the air sacs. After the injection of numerous small doses the capillaries were sometimes tortuous and densely packed with erythrocytes, which occasionally were conglutinated. The walls of the air sacs were somewhat thickened and more cellular, due to proliferating fibrous tissue. The whole picture suggested passive congestion of moderately long standing with diffuse capillary edema; pleural changes did not occur.

The tissue changes produced in the *liver* by arsphenamin are likewise of much interest and practical importance, especially in relation to the occurrence of jaundice. In the experience of Lucke and myself the injection of single large doses have shown extensive and striking changes in all animals, but particularly among the rats; these changes were mainly in the form of extensive areas of coagulation necrosis involving all parts of the lobule, but particularly the central and middle portions. Rats succumbing within two days after the injection have shown changes so extensive as to resemble those commonly found in acute yellow atrophy, entire lobules, excepting the most peripheral portions, being necrotic and sometimes flooded with erythrocytes and infiltrated with mononuclear and fewer polynuclear leukocytes. Among animals surviving a week or longer, the areas of necrosis were not so large. But the injection of ten or more small doses (0.010 gm. per kilo) have shown only in some instances slight periportal fibrosis with small areas of focal necrosis, particularly in the peripheral portions of the lobules, with inconspicuous vascular changes. Probably, of all the tissue changes that may be produced by arsphenamin and its substitutes, most interest has been shown

in the *kidney* lesions as influencing the elimination of the compound and the production of toxic reactions. Pearce and Brown have observed profound changes in dogs and essentially similar changes have been observed by Lucke and myself in the kidneys of rabbits and rats receiving similar single large doses. The vascular changes consisted principally of marked congestion of the vessels of the boundary zone and cortex with occasionally minute interstitial hemorrhages along with cloudy swelling, vacuolization and granularity, involving particularly the convoluted tubules of the inner cortical zone which were sometimes infiltrated with lime salts. *However, numerous small doses have not produced such marked changes* and were more in conformity with the lesions observed in human kidneys. It is well known clinically that lesions of the kidneys are not nearly as likely to develop during arsphenamin therapy as during the administration of mercury and bismuth, and the histological changes found by Lucke and myself after the injection of rats and rabbits with 10 or more doses of 0.010 gm. each per kilo are in conformity with this clinical experience. Vascular changes were sometimes prominent and the tubules have occasionally shown minor changes generally confined to the convoluted tubules of the outer cortical zone and spiral portions of the outer boundary zone but frank necrosis was not encountered and evidences of cellular regeneration were frequently seen.

The effects of arsphenamin upon the *suprarenal glands* are of interest in relation to the possibility of such changes producing disturbances in the adrenalin content of the blood in relation to toxic reactions and especially the immediate reaction or "nitritoid crisis." As previously stated, Pearce and Brown have noted profound changes in glands of guinea pigs and rabbits treated with single large doses (0.100 to 0.150 gm. per kilo) and suggest that therapeutic doses of some arsenicals may produce adrenal stimulation. Lucke and I found that, in practically all rats and rabbits injected intravenously with single doses of this size, great reductions were produced in the lipoid and chromaffin contents but among animals receiving numerous small doses the lipoid and chromaffin substances were apparently unaltered or but slightly decreased.

While arsphenamin produces tissue changes in the *spleen*, these have attracted much less attention being principally due to the possible effects of the drug upon erythrocytes and producing no symptoms. Lucke and I found that in rabbits and rats single large doses frequently produced striking irregular areas of coagulation necroses, but multiple small doses produced much less change, the principle effect being one of passive congestion with slight hemosiderosis and fibrosis, particularly in the animals receiving the maximum number of injections.

Pathological Tissue Changes Produced by Neoarsphenamin.—Neoarsphenamin produces tissue changes in rabbits and rats quite similar to those produced by disodium arsphenamin, but with the exception of the kidney changes, the effects are of lesser severity. Lucke and I have found that the vascular changes are similar, but much less extensive; thrombi of erythrocytes are sometimes found, but since neoarsphenamin does not produce such intravascular changes as agglutination, hemolysis and precipitation, it is likely that these are due primarily to vascular injuries. Cellular degenerations and necroses are likewise to be found in the heart, liver and kidneys, but with the possible exception of the kidneys, are of much lesser severity than produced by arsphenamin. Since the lesions are similar to those described above for disodium arsphenamin, I need not review them here, a detailed description based upon the studies made with rats and rabbits having been made by Lucke and myself.[36]

Most interest is commanded by the histopathological changes produced in the liver and kidneys and the question naturally arises, are the effects produced in these organs by these two compounds of similar kind and equal degree?

As found by Lucke and myself, the pathological changes produced in the livers of rats and rabbits were usually slightly more severe after the injection of arsphenamin than after neoarsphenamin; we did not note any particular differences in effects upon the liver. But Dr. G. C. Lake of the Hygienic Laboratory called my attention to the fact that the kidneys of rats succumbing after intravenous injections of neoarsphenamin prepared by several different laboratories presented a "chocolate" appearance, denoting cloudy swelling and more severe parenchymatous changes than after injections of alkaline solutions of arsphenamin. Hooper, Kolls and Wright also found that neoarsphenamin produced 50 per cent. more injury to the kidneys of rats than arsphenamin, when the effects were evaluated in terms of the amounts of arsenic injected. These investigators also found "arsenoxid" about five times more toxic for rat kidneys than arsphenamin and seven and one-half times more toxic than neoarsphenamin.

Subsequent investigations by Lucke and myself[37] have confirmed these observations, at least to the extent that, when rats were injected intravenously with single large doses of arsphenamin and neoarsphenamin in amounts so arranged that the dose of arsenic per kilogram was approximately the same for both compounds, the arsenic of neoarsphenamin was more toxic for the kidneys than the arsenic of arsphenamin. The arsenic of arsphenamin, however, was more toxic for the liver than the arsenic of neoarsphenamin.

But when arsphenamin and neoarsphenamin were administered to rats in small multiple doses comparable to the amounts ordinarily administered to human adults according to body weight, these special renotoxic and hepatotoxic effects were not so much in evidence. For example, a dose of 0.01 gm. of arsphenamin per kilo carries approximately 0.00031 gm. of arsenic and and 0.02 gm. of neoarsphenamin per kilo about 0.00038 gm. of arsenic; rats receiving 10 to 12 doses of these amounts at intervals of 5 to 7 days did not show in the first place, extensive or very marked histological changes in the liver and kidneys and in the second place, the kind and degree of tissue changes are quite similar for both compounds when these are compared with large numbers of animals kept on exactly the same diet in order to minimize individual variations. In other words, the special hepatoxic effects of arsphenamin and the renotoxic effects of neoarsphenamin are brought out by single large doses of these compounds and probably are due to variations in elimination and storage; with multiple small doses, the toxic effects upon the liver and kidneys are less apparent with both compounds.

The same is true of silver arsphenamin. Kolle[38] states that Fischer has determined that rabbits which had received more than 60 injections of 0.07 gram of silver arsphenamin per kilogram intravenously every second day showed no changes of the organs and Goldstein observed no changes in the nervous system. Schlossberger[39] found in rabbits that received 0.02–0.04 gram silver arsphenamin per kilogram every 3–4 days for 9–12 months, with a total of 3–4 grams, no argyria of the skin. The silver was deposited in the otherwise normal internal organs, especially in the Kupffer cells of the liver as shown by Fischer and to a slight extent also in the kidneys. No functional injury could be demonstrated for the rabbits, which constantly increased in weight.

Hooper and Meyers[40] found that repeated intravenous administration of silver-arsphenamin into rabbits in doses of 10 milligrams or 66.7 milligrams every 3–7 days for a period of 47–70 days did not produce any noteworthy

tissue or blood alterations. In a relative way the amount of silver found on histologic examination of the tissues corresponded with the amount found chemically after incineration. The great individual variation in the amount of silver found at necropsy may have been due to preexisting lesions in the liver and kidneys. In the rabbits that received 10 milligrams silver-arsphenamin per kilogram (3 times the average clinical dose) the silver deposits were inconspicuous.

Pathological Tissue Changes Produced by Arsenoxid, Sulpharsphenamin, Atoxyl and Tryparsamid.—The tissue changes produced by other organic arsenical compounds employed in the treatment of syphilis, trypanosomiasis and other diseases are somewhat similar to those described above for arsphenamin and neoarsphenamin; minor differences are to be noted, but the chief vascular and parenchymatous lesions are similar although varying in severity. For example, *arsenoxid* (para-oxy-meta-amino-phenyl-arsenoxid) produces the same lesions in rats and rabbits as arsphenamin, only more quickly and more intensively. Doubtless, a part of the tissue changes engendered by arsphenamin are due to this oxidation product, although injections of the arsenoxid itself produces greater injury because when arsphenamin is injected a portion is eliminated unchanged before conversion into this trivalent oxid. As previously stated, Hooper and his associates judged that arsenoxid was five times more toxic for rat kidney than neoarsphenamin when compared on an arsenic equivalent basis and seven and one-half times more toxic than arsphenamin (disodium salt). I have observed that *sulpharsphenamin* likewise produces tissue changes similar to those of arsphenamin and neoarsphenamin when injected intravenously in doses corresponding to approximately equal arsenic equivalents; by subcutaneous injection, however, the tissue injuries are less marked although of similar kind.

Pearce and Brown[41] have stated that the subcutaneous or intraperitoneal injection of dogs with *atoxyl*, in dose of 0.020 gm. per kilo, produces parenchymatous and fatty degeneration of the tubular epithelium of the kidneys, with slight congestion and hemorrhage in the boundary zone.

The tissue changes that may be produced by *tryparsamid* (the sodium salt of N-phenylglycine-amide-p-arsonic acid) have been carefully studied by Brown and Pearce,[42] who state that the changes seen in the early stages of the intoxication following large doses in the lower animals consist in moderate vascular dilatation and congestion with a few scattered petechial hemorrhages, occasional effusions into the serous cavities, and widespread cellular degenerations or even necrosis in some organs. The organs mostly involved are the kidneys and adrenals, the cardiovascular system, with an uncertain involvement of the central nervous system, the gastrointestinal tract, the blood, and blood-formative organs; the changes produced in other organs have appeared to be of minor importance. Animals which survived the intoxication rarely showed lesions in organs other than the kidneys and myocardium and doses of the drug slightly below the level of the lethal dose produced only very slight tissue injury. For detailed descriptions of these various organic changes produced by tryparsamid, the reader should consult the publications by Brown and Pearce.

Clinical Significance of the Chemopathological Tissue Changes Produced by Arsphenamin and Its Substitutes; the Toxic Reactions Due to Arsenic.—We may now properly inquire into the clinical and practical significance of these pathological tissue changes which may be engendered by arsphenamin, neoarsphenamin and other organic arsenical compounds in the lower animals in relation to the treatment of syphilis and other diseases of human beings. Upon hasty and superficial examination of the preceding paragraphs concern-

ing these pathological changes, one may readily enough acquire the impression that the drugs are capable and indeed likely, of producing such wide-spread and severe lesions as to preclude their administration in the treatment of disease. But it is to be remembered that the severe lesions were produced especially by very large single doses, amounts almost lethal and ten times or more larger than the amounts given human beings at one time in proportion to body weight, whereas with doses approximately similar to those given in the treatment of syphilis, the tissue changes were much less marked and frequently negligible. In other words, single large doses bring so much of the compound in relation to the body cells, that the tissue affinities and pathological lesions are intensified before elimination and reduction to less harmful substances can occur; with numerous small doses, however, a portion of the compound is quickly eliminated and the cells thereby spared even though the total amount administered in this way over weeks of time may be greater than that given in a single injection.

Chemopathological studies of this kind are of great value in the interests of chemotherapeutic investigations. They have shown, for example, that even though toxic symptoms are not produced or may escape detection, that significant tissue changes may occur. They should form a part of every thorough chemotherapeutic investigation before a new compound is endorsed for administration to human beings and now that an increasing number of drugs are being given by intravenous injection, it is advisable that pathological tissue studies of this kind should be included more generally in pharmacological investigations. But I have already discussed this subject and only mention it here again to lay proper emphasis upon the significance and clinical value of chemopathological investigations.

As previously stated, the pathological changes observed after the administration of arsphenamin, neoarsphenamin, atoxyl, etc., are mostly ascribed to the effects of ionic arsenic, but we do not know from what organic combination this free arsenic is mostly produced. Doubtless the trivalent oxids resulting from oxidation or reduction are an important source, since the injection of arsenoxid is known to be many more times toxic than arsphenamin and neoarsphenamin, but it is entirely likely that the arsenic as stored (presumably in a pentavalent form) is likewise toxic but to a much lesser degree. Doubtless most if not all the tissue injuries produced by the administration of the organic arsenicals are caused by ionic arsenic derived not only from the compounds themselves but also from arsenical impurities in commercial arsphenamin and neoarsphenamin; but concerning these we have much less information.

At any rate, we may safely conclude that most of the tissue changes are due to some form of arsenic, and the outstanding changes are, first an effect upon the capillaries characterized by relaxation and congestion with serous exudation and minute hemorrhages which is a characteristic tissue change of arsenic in general; followed, secondly, by parenchymatous degenerations and necroses. It is entirely likely that the "nitritoid crisis" or immediate toxic reaction of arsphenamin and neoarsphenamin is due primarily to this direct action of arsenic upon capillaries, but in individuals with hypersensitiveness to arsenic, since the effects may be produced by amounts without effect upon the majority of individuals. Likewise, the skin eruptions (urticaria, exfoliative dermatitis, etc.,) may be due to the direct toxicity of arsenic upon capillaries and the excretory glands of the skin, while jaundice is to be ascribed primarily to the necrosis of liver cells by arsenic or to an exacerbation of syphilitic lesions in the liver in the nature of hepatic Herxheimer reactions.

A sharp distinction should be made, however, between the pathological tissue changes produced by single, large, toxic doses of a compound and those that may be produced by multiple, small, clinically non-toxic and therapeutic sized doses; both, and especially the former, are of interest to the pharmacologist and experimental chemotherapeutist, but only the latter are of direct clinical interest in relation to the treatment of disease.

As shown by Lucke and myself, the tissue changes produced in rats and rabbits by ten intravenous injections of arsphenamin in alkaline solution in dose of 0.01 gm. per kilo corresponding to 0.6 gm. for an adult of average weight, are usually inconspicuous and probably without clinically discoverable effects upon the physiological functions of the involved organs; the same is true of neoarsphenamin in dose of 0.02 gm. per kilo, which corresponds to about 1.2 gm. for an average human adult. I believe it may be said, therefore, that the intravenous injection of these compounds every three to seven days in the doses mentioned do not produce tissue changes of sufficient degree and importance materially to influence the physiological functions of the majority of individuals and an enormous number of injections to human beings the world over during the past fifteen years, since these compounds came into wide and general use in the treatment of syphilis, affords clinical confirmation of this assertion. It is true that albuminuria sometimes develops during the treatment of syphilis with these compounds, but not as frequently as heretofore surmized, and indeed, albuminuria due to syphilitic infection or the administration of mercury, is very likely to disappear under arsphenamin and neoarsphenamin treatment.

Probably the toxic effects of arsphenamin and its substitutes upon the kidneys and liver have attracted most attention from the clinical standpoint, and the importance of frequent examinations of the urine for albumin and casts is not to be underestimated; but I believe it may be said with conviction that arsphenamin and neoarsphenamin are not particularly renotoxic and certainly much less so than mercury. As stated above, neoarsphenamin is more apt to injure the kidneys of rats than arsphenamin, but, with the usual small doses employed in the treatment of syphilis, the kidney changes are apparently slight with both compounds and almost neglible under ordinary conditions. For example, the investigations of Rappleye,[43] Elliott and Todd,[44] Bailey and MacKay,[45] Anderson,[46] Weiss and Corson,[47] Raiziss and Brown[48] and others upon disturbances of renal function in human beings or rabbits, as determined by renal functional tests or blood chemical determinations for urea and total non protein nitrogen, creatinine, etc., have shown that multiple, small, therapeutic sized doses of arsphenamin and neoarsphenamin have no injurious effects upon renal function, insofar as these may be ascertained by tests of these kinds. With single, large, toxic doses marked disturbances may occur and I may mention in this connection that Raiziss and Brown found that neoarsphenamin was less likely to disturb the renal function of rabbits than arsphenamin which is not in agreement, therefore, with the results of the histological studies mentioned above, showing that neoarsphenamin was more toxic for the kidneys of rats when administered on the basis of arsenic equivalent per kilo of weight. It cannot be overlooked, however, that both drugs may produce severe types of nephritis in some cases of syphilis and especially if the organs are previously damaged by infection or drugs and particularly by mercury. Probably under these circumstances the more severe effects are due to interference with permeability and elimination with consequent retention of arsenic and other injurious derivatives; in other words, the effects of arsphenamin and its substitutes upon damaged kidneys is apt to be a different matter from effects upon normal kidneys, although in the

presence of syphilitic nephritis improvement rather than further damage is apt to result from judicious medication with these compounds. I believe, however, that in nephritis it is better practice to administer arsphenamin than neoarsphenamin in view of the apparent greater toxicity of the latter for the kidneys.

The same may be said regarding the clinical effects of arsphenamin and neoarsphenamin upon the liver; that is, ordinary, therapeutic-sized doses properly administered do not produce clinically discernible changes in this organ of approximately normal individuals; although in syphilitic or other types of hepatitis, severe effects may be produced. Unfortunately, we do not yet possess tests for liver dysfunction of equal value to the tests for kidney dysfunction in present use, but the liver functional tests so far developed do not show that arsphenamin and neoarsphenamin are particularly hepatotoxic in the usual doses. Greenbaum and Brown[49] for example, found that 75 per cent. of syphilitic patients treated with as many as 45 to 124 intravenous injections of neoarsphenamin showed no evidences of liver dysfunction, as determined by the phenoltetrachlorphthalein test of Rosenthal;[50] it should be stated, however, that this test likewise showed no evidences of liver dysfunction in patients developing acute toxic reactions, although among cases developing jaundice, the test yielded evidences of functional disturbances of the liver which tended to persist even after clinical recovery. Greenbaum and Brown believe, however, that the test may be of value in cases receiving arsphenamin or neoarsphenamin showing signs and symptoms of toxic reactions; Piersol and Bockus[51] have likewise stated that the test as conducted by them possessed diagnostic value for impairment of liver permeability, but Einhorn[52] and Hoxie[53] have not found it sufficiently sensitive and accurate and in all probability it fails to show impairment of permeability in the presence of minor degrees of focal parenchymatous changes as may be produced by small amounts of arsphenamin and its substitutes. Possibly the so-called "hemoclastic crisis" test of Widal, Abrami and Iancovesco[54] and tests for bilirubinemia as described by Bernheim,[55] may yield additional information, but no statement can be made at present of their value in relation to the liver injuries that may be produced by the organic arsenical compounds. At any rate, the treatment of cases of syphilis showing signs and symptoms of hepatitis with arsphenamin and its substitutes involves greater risks and demands extraordinary care; under these circumstances preference is to be given neoarsphenamin, since it is apparently less toxic for the liver than arsphenamin.

The question is frequently asked whether or not arsphenamin and neoarsphenamin produce more or less permanent injuries of the heart and blood-vessels. I believe that, with the usual therapeutic-sized doses, it may be concluded from histological studies among the lower animals that such do not occur. It is possible, as will be shortly discussed, for these drugs to intensify lesions in the cardiovascular system as elsewhere in the way of producing Herxheimer reactions, but I doubt if the drugs alone contribute in any important manner to the production of chronic myocarditis, aortitis, hypertension, etc., of syphilitic individuals undergoing arsenical treatment.

However, it is to be remembered that, among the earliest and most constant tissue effects of the arsenical compounds in general, is an action upon the blood-vessels and especially the capillaries in the form of vasomotor paralysis and endothelial necrosis. Among individuals with congenital or acquired hypersensitiveness to the arsenic of these drugs, immediate vasomotor disturbances are likely to develop after administration in the form of flushing, serous exudation, fall of blood-pressure, etc., constituting the

"nitritoid crisis." While the symptoms and lesions of this acute reaction may be caused by other factors, I believe that in some cases at least, the effects are due primarily to hypersensitiveness to arsenic in which the vasomotor changes are in part due to allergic shock and in part to the direct effects of arsenic upon the blood-vessels. When involving the vessels of the meninges, acute cerebral symptoms may be produced, due to increased cerebrospinal fluid pressure from the serous exudation and minute hemorrhages may result, constituting the so-called "hemorrhagic encephalitis" so prominently mentioned in the German literature as a cause of arsphenamin fatalities. According to the results observed by Lucke and myself in rabbits and rats, ordinary small doses of arsphenamin and neoarsphenamin do not produce prominent changes in the brain and meninges and it is a peculiar fact that in the United States deaths ascribed to arsphenamin and neoarsphenamin have not been regarded as caused by this type of lesion properly designated as acute meningeal congestion with serous exudation and hemorrhage.

The effects produced in the adrenal glands are likewise of interest, although these are inconspicuous in normal animals treated with multiple, small, therapeutic sized doses. But since the acute immediate "nitritoid" reaction bears so many clinical similarities to the lesions and symptoms of acute adrenal insufficiency as originally emphasized by Milian[56] and since the subcutaneous injection of adrenalin affords such prompt relief, one can hardly escape suspecting that the effects of arsphenamin in particular upon the suprarenal glands may be a matter of importance. Hirano[57] has indeed described a deficiency of adrenalin in the blood after injections of arsphenamin, but in the experiments by Lucke, McCouch and myself[58] these finding were not confirmed. The mere fact that the subcutaneous injection of adrenalin prevents or relieves the "nitritoid" reaction is no indication that this toxic reaction is due to adrenal insufficiency; the "nitritoid crisis" is due to acute vasomotor paralysis and this may be caused by the effects of arsenic upon the capillaries and especially if allergic sensitiveness to arsenic exits. In any event, the injection of adrenalin may be expected to afford relief by producing vaso-constriction as it does in allergic reactions and other types of acute vascular congestion. I believe that certain vagotonic individuals may owe their state to adrenal insufficiency and that among these the occurrence of "nitritoid crises" may be unusually high, but I am not at all convinced that the effects produced by ordinary doses of arsphenamin and neoarsphenamin upon the adrenal glands are primarily responsible for these reactions.

So far our discussion has been largely based upon the tissue injuries that may be produced by arsphenamin and its substitutes in approximately normal individuals. But how may these be modified by the presence of syphilis or other infections with resulting pathological changes? In the majority of cases of early primary syphilis, the individuals are otherwise healthy and widespread pathological changes have not been produced by the spirochetes so that the patient may be regarded from this standpoint almost in the light of a normal individual; but in late syphilis the situation may be entirely different. In such we may have organs already damaged and if the liver and kidneys are sufficiently involved to interfere with permeability and elimination, more intense tissue injury may be produced by the injudicious injection of arsenical compounds. Even focal infections ordinarily regarded as of minor importance, may be sufficient for intensifying toxic reactions as so ably discussed by Duke[59] and Stokes,[60] and the influence of previous disease upon the tissue injuries and toxicity in general of arsphenamin and its substitutes becomes a far more important matter than the effects in normal individuals.

It is possible for the medicaments to aggravate syphilitic processes (Jarisch-Herxheimer reactions) and thereby produce lesions and effects not observed in non-syphilitic individuals, and it may be that under these circumstances, further dissemination of the spirochetes is favored by reason of the marked vascular changes of these reactions. It is not difficult to understand that, in organs already the seat of inflammatory changes, the vascular and parenchymatous changes produced by arsenic or other constituents of the organic arsenical compounds may be intensified and especially of the liver, in which arsenic tends to become stored.

In other words, toxic reactions following the administration of arsphenamin and other organic arsenical compounds may be due to faults in the individual patient and some of these may readily enough intensify the pathological tissue changes, but this subject is so broad and important that I will leave further discussion of it for Chapter XLVII. Here, I believe, it may be conservatively stated that amounts of arsphenamin, neoarsphenamin, etc., insufficient for producing pathological tissue changes in normal individuals, may be capable, however, of doing so in diseased individuals and particularly those with advanced chronic lesions of syphilis; for this reason the treatment of cases of chronic syphilis usually presents more and different problems than encountered in the treatment of cases of early syphilis.

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CHAPTER XLVII

ABNORMAL CHANGES IN THE PATIENT IN RELATION TO TOXIC REACTIONS FOLLOWING THE ADMINISTRATION OF ARSPHENAMIN, NEOARSPHENAMIN AND OTHER ORGANIC ARSENICAL COMPOUNDS

We may now properly inquire into the relation syphilis itself and other pathological states of individual patients bear toward the etiology of various reactions sometimes following the administration of arsphenamin and its substitutes and especially the more severe and more dangerous reactions. The natural tendency is first to blame the drug and technic of administration, but it need scarcely be mentioned that reactions develop even with the best of compounds administered in an apparently correct manner, and not infrequently the primary cause is to be found in the patient himself.

Fear, Nervousness and Endocrine Disturbances in Relation to Untoward Reactions.—In the first place, plain fear and nervousness are alone sometimes responsible for nausea, vomiting and syncope. I have frequently observed these symptoms during the introduction of the needle and before a single drop of solution of drug has been injected and, curiously enough, men are more likely to show these symptoms than women. As a general rule, this fear and nervousness is dispelled after the first injection or two when the patient is reassured that there is nothing to fear.

But individuals of both sexes with unstable nervous systems, those who blush easily, are readily excited, who develop urticaria on frequent occasions, who present vagotonic symptoms and who have other diseases due in part to instability of the nervous system as hay-fever, vasomotor rhinitis and allergic dust asthmas, are generally more prone, in my experience, to develop minor reactions after intravenous injections of arsphenamin and neoarsphenamin than normal persons. The arsenic of these compounds tends to produce capillary congestion by direct action upon the vessels or upon the vasomotor nerves

and in states of general nervous instability this tendency is apparently increased. It may well be that disturbances of some of the endocrine glands may bear a relation to this subject, but in my opinion these glands are not primarily involved by the drugs; rather there is a pre-existing fundamental change due to other causes which in turn may predispose or intensify the usual or what may be called, the normal or physiological effects of arsphenamin and its substitutes. The acute vasoparetic reaction or "nitritoid crisis" is regarded by some as due to sudden insufficiency of adrenalin in the blood through an effect of the drugs upon the suprarenal glands, but, as discussed in Chapter XLVI, I believe the evidence offered in support of this hypothesis is quite inconclusive and that the reaction is due primarily to an exaggeration of the physiological effects of arsenic upon blood-vessels and especially the capillaries. Even though the administration of adrenalin acts as a specific in the treatment of this particular reaction, it is no argument in itself that the drugs may produce sudden adrenalin insufficiency; adrenalin acts simply as a vasoconstricting agent. Disturbances of the thyroid gland may also act as a predisposing factor and some physicians claim that the generally higher incidence of untoward reactions in women may be due to such as well as to disturbances of ovarian secretions; but our knowledge of the whole subject at present is very indefinite in relation to toxic reactions following the administration of arsphenamin and its substitutes, and at most one can but state that such predisposing factors may exist in some patients.

Focal Infection in Relation to Toxic Reactions.—It is well known that infected gums (pyorrhaea alveolaris) greatly predispose to gingivitis and stomatitis from the administration of mercury and bismuth in the treatment of syphilis; this is due primarily to the fact that both of these drugs are eliminated in part in the mouth and tend to become deposited in these tissues where inflammatory phenomena are excited not so much by local irritation through the excreted or deposited compounds as by reason of these lowering local resistance to microbic infection. Does arsphenamin and its substitutes lower resistance to microbic infection? May some toxic reactions result from exacerbations of microbic infections as the direct or indirect effects of these organic arsenical compounds? Do microbic infections reduce tolerance for the organic arsenical compounds employed in the treatment of syphilis?

In seeking answers to these questions one may first enquire if arsphenamin and neoarsphenamin depress antibody production and the functions of the bone marrow and other antibody producing tissues. In 1918, Toyama and myself[1] showed that small doses of arsphenamin, comparable to those commonly administered in the treatment of syphilis, did not appear to depress antibody-production in rabbits, and similar results have been recorded by Dr. Ainslee Walker of England. But it is likely that numerous large doses of arsphenamin or neoarsphenamin in rare and exceptional cases may produce histological changes in the bone marrow of some human beings, as shown by studies of the leukocytes of the blood reported by Moore and Keidel[2] sometimes amounting to severe aplastic anemias, and thereby reduce resistance to bacterial infection, since the bone marrow is recognized as an important organ of antibody-production, as indicated by the investigations of Hektoen[3] and others. Stokes and Ruedemann[4] have shown that the surgical interference of foci of infection in the tonsils, apices of the teeth and elsewhere during arsphenamin treatment may precipitate severe and even fatal attacks of dermatitis, not so much by the local infection of the skin with bacteria from these foci, as by favoring the production of bacteremia or of allergic sensitization of the skin with the proteins of these organisms.

Moore and Keidel have expressed a similar view in that allergic sensitization to bacterial proteins may occur in cases of focal infection and that the drugs, by reason of the vasodilating properties of the arsenic, bring added amounts of protein to the skin where various dermatoses may be excited. I shall discuss these observations and views with more detail in the succeeding chapter in the section devoted to the etiology, symptoms and treatment of dermatitis and allied reactions, but here I believe one may conservatively state that focal infections, and especially the more severe and advanced grades, are worthy of serious consideration in the treatment of syphilis as a possible predisposing factor to arsenical dermatitis. As shown by Stokes,[5] for example, focal infection may reduce the tolerance of the kidneys for arsphenamin and Duke[6] has shown that focal infection may intensify the lesions of neurosyphilis.

Furthermore, since the liver is recognized as an important depot of storage of arsenic as well as of its elimination, it is not at all unlikely that bacterial infection of this organ and its bile-ducts may be intensified by the arsenic and thereby result in the production of jaundice, not so much the result of direct toxic hepatitis by the drugs as the acceleration of infection of the organ by a lowering of local resistance. The same may be true in cases of intestinal infections, since arsenic is eliminated by the mucosa with the production of vascular changes of hyperemia and exudation.

Arsphenamin and its substitutes may, therefore, in exceptional cases at least, favor the production of dermatitis and jaundice by lowering resistance to bacterial infection or in some other manner stirring them into greater activity, and for this reason the subject of focal or frank infections of individual cases is deserving of due consideration in the preparation of patients for the treatment of syphilis and for added care in our dosage and general treatment.

Syphilis in Relation to Toxic Reactions.—Does the kind and severity of syphilitic lesions bear any relation to the incidence and variety of toxic reactions following the administration of arsphenamin and its substitutes? It is well known that animals infected with virulent trypanosomes, as *Tr. equiperdum*, are much less resistant to the toxic effects of arsphenamin and neoarsphenamin than normal healthy animals. Yakimoff and Yakimoff[7] have found this true of rabbits infected with trypanosomes and I have found it constantly true of infected rats and to a lesser extent with syphilized rabbits. It may be that this increased toxicity of arsphenamin and its substitutes for infected animals is due to a summation of the pathogenic effects of the parasites and the toxic effects of the drugs, but on the other hand, the increased toxicity may be due in part to the liberation of toxic substances by the action of the drug upon the parasites. McIntosh, Fildes and Dearden[8] and others have stated that the fever and rigors so frequently following the administration of arsphenamin to cases of syphilis in the secondary stage are due to liberated "endotoxins" from *Spirochæta pallida* and I believe it may be stated without reserve that mild reactions in all stages of syphilis, but especially in the acute early stages when spirochetic infection is numerically heavy, may be due in part to the liberation of toxic factors from organisms destroyed by the medicament employed and that in a broad and general manner individuals *acutely* infected with *Spirochæta pallida* and, indeed, with other organisms, are more likely to develop untoward reactions than healthy human beings. This is due not only to the possible liberation of toxic substances from parasites, but in large part to a general reduction of resistance to the toxic effects of the drug. Of interest in this connection is the report of Meirowsky and Kretzmer[9] in their excellent monograph in 1914 on the

"Salvarsan Therapy of Syphilis," of the 109 fatalities following the administration of salvarsan up to that time in which it was recorded that 41 per cent. occurred in the secondary period and 27 per cent. in late nerve syphilis. Three-fifths of the fatalities during the secondary stage was attributed to "encephalitis" and it is not unlikely that this was due to the combined vasculotoxic effects of the arsenic and the liberation of toxic substances from spirochetes in the meninges and brain.

Furthermore, it is well known that the administration of mercury or arsphenamin and its substitutes may produce an exacerbation of syphilitic lesions and especially in the secondary stage as originally described by Jarisch, Herxheimer and Kraus; these effects are probably due in part at least to the liberation of toxic substances from destroyed spirochetes and when occurring in the meninges, may produce very dangerous reactions indeed as just referred to above.

There is, therefore, a possible relation between the acute early lesions of syphilis and the occurrence of toxic reactions from arsphenamin and its substitutes. This may not be anything more than a general lowering of resistance from the effects of the infection but in addition, there may be the added effects of liberated toxic substances from destroyed parasites analagous to the very definite lowered resistance of infected rats and rabbits to these compounds. The importance of the influence of acute syphilis upon toxic reactions, however, should not be over-emphasized; indeed, in most instances the incidence of reactions does not appear to be higher than usual in secondary syphilis, except possibly, for a higher incidence of fever and mild chills.

But in chronic syphilis I believe that the lesions may bear a relation to toxic reactions and especially syphilis of the liver in relation to jaundice if the involvement is severe enough to interfere with the functions of this organ in the metabolism and elimination of the compounds. In a strict sense, the stimulation of syphilitic foci in the liver by treatment (hepatic Herxheimer reaction) is not a toxic reaction on the part of the drug; neither is the exacerbation of liver syphilis after the cessation of treatment (hepatorecurrence) and both of these reactions cover the majority of cases of jaundice in relation to syphilis itself, although I cannot dismiss the possibility that syphilitic hepatitis may predispose to jaundice and that in some cases of tertiary syphilis with sub-icteric skins and mucous membranes, the jaundice that appears after several doses of arsphenamin or neoarsphenamin is due to a combined syphilitic and arsenical hepatitis rather than a syphilitic hepatitis alone in the form of a hepatic Herxheimer reaction; Stokes and Ruedemann, however, state that in their series of cases of jaundice, the instrumentality of syphilis was almost negligible.

Likewise in arsenical dermatitis one may surmise that the capillary endothelial injuries produced by *Spirochæta pallida* in the early stages of syphilis may predispose to the deposition of arsenic in the skin with the production of various dermatoses; but such does not appear to be the case. In the series of dermatitis reported by Moore and Keidel only two occurred among 1800 early cases of syphilis (1 in 900 cases).

In chronic syphilis, however, and especially in cases with sufficient involvement of the liver and kidneys to interfere with the metabolism and elimination of arsenic, it is not unreasonable to surmise that the skin may be irritated by excessive amounts of arsenic brought to it for elimination and resulting in the production of vascular lesions as urticaria and fleeting erythemas by intensifying the physiological effects of arsenic upon capillaries in addition to direct irritation. In my experience the majority of the mild

and severe dermatoses associated with the administration of arsphenamin, neoarsphenamin and sulpharsphenamin have occurred among cases of chronic latent and active syphilis and 15 of the cases reported by Moore and Keidel occurred among 7200 late cases of syphilis (1 in 480 patients), although they are of the opinion that the stage of syphilis bears no reaction to the incidence of dermatitis and allied reactions following the administration of arsphenamin.

It is, therefore, extremely difficult to properly evaluate the influence excited by pathological tissue changes produced by *Spirochæta pallida* upon the occurrence of toxic reactions following the administration and especially the intravenous injection, of arsphenamin and its substitutes. One may easily conjecture at great length but with little profit, in view of the very unsatisfactory and incomplete state of our knowledge of the etiology and mechanism of these reactions. I believe, however, that it may be granted in a broad and general manner that chronic syphilitic lesions, and especially of those organs as the liver, kidneys and bone marrow concerned in the metabolism of the organic arsenicals, may exert some predisposing etiological influence at least and that, when such lesions are known to be present or suspected, our choice of remedy and its dosage should be influenced thereby in efforts toward the prophylaxis of toxic reactions.

Toxic Reactions in Non-syphilitic Individuals.—It is well known that the administration of arsphenamin and neoarsphenamin to apparently healthy individuals or individuals with non-syphilitic diseases may produce toxic reactions exactly similar to those observed among syphilitic patients, at least insofar as the milder reactions are concerned. Observations of this kind have established that the milder reactions cannot be ascribed solely to the release of toxic substances from spirochetes by the drugs administered, but that other factors are responsible operative alike in both syphilitic and non-syphilitic individuals.

But may the presence of syphilis act in the reverse manner and actually increase tolerance for arsphenamin and its substitutes in such manner that the incidence of reactions is lower among those with syphilis than among non-syphilitic individuals? Not infrequently one observes that actively syphilitic individuals withstand much larger doses of mercury and iodid than the average normal non-syphilitic individual and I believe the same may be true in relation to arsphenamin and neoarsphenamin. I have the distinct impression that non-syphilitic individuals are more apt to develop milder reactions than those who are actively syphilitic, although I am unable to compare the two groups in relation to the severer reactions as exfoliating dermatitis and jaundice, since I have never given non-syphilitic individuals more than two injections of either drug except in cases of pernicious anemia and in a few other diseases in whom pathology states existed which may have influenced the reactions.

Jarisch-Herxheimer Reaction in Relation to Toxic Reactions.—Of further interest in connection with the subject of syphilis in relation to toxic reactions by arsphenamin and its substitutes and of great practical importance in the diagnosis and treatment of toxic reactions, are the Jarisch-Herxheimer reactions sometimes observed after the first dose or two of these drugs.

In 1895, Jarisch[7] first described a series of cases of secondary syphilis in whom the roseolar eruptions became intensified after 2 to 5 inunctions of mercury and seven years later Herxheimer and Kraus[8] greatly renewed interest in the subject by describing the exacerbation of these lesions by the injection of the first large dose of an insoluble salt of mercury. In 1910 Finger[9] described the exacerbation of the rashes of the secondary stage of

syphilis following the administration of arsphenamin and the reaction is now well known as the "Jarisch-Herxheimer reaction."

These reactions have been observed, therefore, not only after the administration of mercury but also after arsphenamin, neoarsphenamin and bismuth. It is commonly believed that they are not due to the direct toxicity of the drugs, but rather to the effects of endotoxins liberated from destroyed spirochetes. With arsphenamin and its substitutes, however, this may not be the complete mechanism, since it appears that the vasculotoxic effects of arsenic upon the capillaries may play a part in their production. This subject is discussed at further length in Chapters LI and LVIII, but is mentioned here in relation to the influence of syphilis upon the incidence of toxic reactions following the administration of the organic arsenical compounds and especially since similar reactions may occur in organs other than the skin. As just stated, both Jarisch and Herxheimer confined their observations to the influence of mercury upon the acute syphilitic exanthems, but since then and especially since the introduction of salvarsan in the treatment of syphilis, the phrase "Jarisch-Herxheimer reaction" has been applied to the exacerbation of chronic syphilitic lesions in the liver, cardiovascular and central nervous systems, etc., after the institution of specific treatment.

As a general rule, the reactions are not difficult of diagnosis, except when occurring in the liver; the resulting jaundice is frequently difficult of proper classification as discussed at length in Chapter LI, because it may be due to syphilis alone, to an exacerbation of syphilitic hepatis, to the drug alone or to a combination of syphilis and drug. Obviously, however, Jarisch-Herxheimer reactions are of great importance from the standpoint of the treatment of syphilis; apparently they may be prevented by instituting specific treatment mildly rather than intensively, which may be important especially in the treatment of chronic syphilis involving the optic and auditory nerves and similar physiologically important tissues. Their diagnosis is also important because treatment consists in principle of the continuation of the administration of spirocheticidal agents rather than the cessation of treatment as in the case of true toxic reactions.

Syphilitic Recurrences in Relation to Toxic Reactions.—Brief mention may be also made here of the subject of recurrences of spirochetic activity after the cessation of the treatment of syphilis by arsphenamin and its substitutes, since these may be mistaken clinically as delayed toxic reactions. The subject is discussed with more detail in Chapter LVIII.

Sometimes it is noted that, during a course of injections of arsphenamin and neoarsphenamin, no untoward reactions occur or but the mild and usual reactions. A week or two later, however, reactions occur in the form of jaundice, a disturbance of sight, hearing, etc. It is believed that in some instances these reactions are due to a re-activity of syphilitic processes rather than the result of toxic effects of the drug upon the tissues concerned; for this reason they are called "syphilitic recurrences" or "syphilitic recidives" and are sometimes designated according to the organ involved as "hepato-recidive," "meningo-recidive," "optic recidive," etc. Obviously they are of great importance from the standpoint of diagnosis and treatment because in principle they may demand even more rigorous specific medication rather than its interruption as in the case of true toxic reactions.

Non-syphilitic Diseases in Relation to Toxic Reactions by Arsphenamin and Its Substitutes and Contra-indications to Their Administration.—And the question now arises regarding the influence of pathological changes due to non-syphilitic diseases upon the incidence and variety of toxic reactions following the administration of arsphenamin, neoarsphenamin and their

substitutes. Very early in the history of the treatment of syphilis with arsphenamin is to be found the warnings of Ehrlich, Wechselmann and other pioneers against the indiscriminate use of the drug in cases of advanced cardiovascular disease, nephritis, etc.

Pathological changes in the physiologically important organs the result of syphilis, of non-syphilitic infection, drugs as mercury or other causes are important in relation to the toxic effects of the medicinal organic arsenicals in two important ways, namely, (*a*) by the possibility of intensifying the physiological effects of the drugs until they reach an abnormal or pathological degree and (*b*) by interfering with the metabolism of the drugs and the elimination of their end products some of which are apparently toxic and capable of harm if retained too long or are forcibly eliminated by unusual channels.

When these drugs are administered there is almost bound to be a primary increase of blood-pressure before the secondary fall due to the effects of arsenic, by the excitement attending the injection, aided especially in the case of arsphenamin, by the injection of 100 to 300 c.c. of fluid. In advanced cardiovascular disease this sudden rise may be injurious and especially in advanced myocardial involvement, aneurysm, pulmonary tuberculosis with hemoptysis, etc. Furthermore, the vasodilating effects of the arsenic may be dangerous in cases of incipient hemorrhage and the effects of arsphenamin and especially of neoarsphenamin upon lowering the coagulation and bleeding time of the blood may constitute a contra-indication to the use of compounds of this class in hemorrhagic diseases as hemophilia.

Advanced disease of the liver and kidneys likewise call for caution in the administration, not only of arsphenamin and neoarsphenamin but of mercury and bismuth as well, in view of the fact that these organs are so intimately concerned in the elimination of these compounds. It is not to be inferred that the presence of chronic nephritis, or of such symptoms as lead to the suspicion of involvement of the liver and its bile-ducts, constitutes a contra-indication to the use of antisyphilitic drugs; indeed, the very reverse may be true if these lesions are believed to be actually the effects of syphilitic infection. But the presence of lesions of these organs does demand more than the usual care in the selection and administration of remedies in that arsphenamin or neoarsphenamin are to be preferred to mercury and bismuth in the presence of nephritis, because these are less renotropic; while in hepatic disease probably bismuth is to be preferred, because less hepatotropic or arsphenamin in preference to neoarsphenamin, since the former appears to be less hepatotoxic than the latter. And what is more important still, the dosage must be regulated accordingly under the general principle that small frequently repeated doses may be well borne and exert curative effects, whereas large doses may be harmful. This is especially true in the treatment of advanced syphilitic infection of the heart and aorta and is likewise true in the treatment of syphilis of those individuals with syphilitic or non-syphilitic cardiac, renal and hepatic disease.

There are, therefore, very distinct contra-indications to the usual doses of arsphenamin (0.6 gm.) and neoarsphenamin (0.9 gm.) in the form of advanced syphilitic and non-syphilitic diseases as hemophilia, diseases of the endocrine glands such as deficient functioning of the thyroid gland, as stated by Lortat-Jacob,[10] exophthalmic goiter and Addison's disease, advanced cardiovascular disease, severe pulmonary tuberculosis, advanced and acute nephritis, hepatitis and gastro-intestinal catarrh, acute septic infections and constitutional tendency to skin diseases, either because these dangerously intensify the physiological effects of these drugs or dangerously interfere with the elimina-

tion of their products; but in practically all of these conditions smaller doses as 0.2 gm. or less of either may be given with comparative safety, if the presence of syphilitic lesions demands in the interests of the life and welfare of the individual that specific medication shall be given. I repeat as my conviction that the only absolute contra-indication to the administration of arsphenamin and neoarsphenamin from the standpoint of toxic reactions is arsenical hepatitis, provided that small doses are given (0.2 gm. or less), although the list of contra-indications to the usual large doses is by no means small or negligible. The list of contra-indications to single large doses, and especially large first doses, may be extended by including those syphilitic lesions in which sudden exacerbations (Jarisch-Herxheimer reactions) may be dangerous or disastrous; these are fully considered in Chapter LI, but here again it may be stated that even these are avoidable by a conservative attitude on the part of the physician and adherence to the principle of starting with small gradually increasing doses rather than the administration of large doses at the beginning of the treatment of those cases of syphilis in whom these reactions, if they occur, may be dangerous.

Administration of Mercury and the Iodids in Relation to the Production of Toxic Reactions by Arsphenamin and Neoarsphenamin.—Of special interest, however, in relation to the influence of non-syphilitic diseases upon the incidence and severity of toxic reactions from the administration of arsphenamin and its substitutes, is the effect of the previous or simultaneous administration of mercury. Wechselmann[11] very early expressed the opinion, after critically reviewing the fatalities ascribed to arsphenamin from 1910 to 1913 including one case of his own, that nephritis due to mercury greatly increased the danger of toxic reactions by salvarsan presumably by interfering with the elimination of the drug with the consequent production of oxidation products of increased toxicity of which Ehrlich's "arsinoxid" was mentioned as a type. Schamberg[12] in this country has recently subscribed to the same view and believes that the large number of cases of delayed arsenical poisoning reported by Strathy, Smith and Hannah[13] may have been due to the fact that intensive treatment with mercury accompanied the injections of neoarsphenamin and especially since the authors themselves were of this opinion, as they state: "A few of the patients showed slight symptoms of mercurial poisoning, but mercury is known to be an irritant of the kidneys and in excessive doses to cause degeneration of the tubular epithelium, and it seems not at all unlikely that for this reason it acted as a predisposing factor."

Like in everything else, a conservative and well-balanced attitude is required in regard to this question of the influence of mercury upon the production of toxic reactions by the organic arsenicals. I am absolutely sure that mercury can be given before or during treatment with arsphenamin or neoarsphenamin without injury; on the other hand, I am equally sure that the administration of the usual doses of arsphenamin, neoarsphenamin and sulpharsphenamin in the presence of mercurial nephritis, as indicated by the presence of albumin and casts in the urine and possibly associated with the retention of urea and other nitrogenous products, may be fraught with danger and requires more than usual caution in dosage. Certainly there can be no reasonable doubt of the fact that injury of the kidneys by mercury, focal infection or any other agent to the extent of appreciably reducing the elimination of arsenic increases the danger and incidence of arsenical injuries; tying off the ureters greatly increases the toxicity of arsphenamin and other organic arsenical compounds, as shown experimentally by Voegtlin and his associates. But recent writers are not giving this subject of the influence of

mercury upon the toxic arsphenamin reactions as much emphasis as earlier writers. For example, Stokes and Ruedemann were unable to work out any relation between the administration of mercury and the incidence of jaundice and Moore and Keidel could observe no influence of mercury upon the incidence of arsphenamin dermatitis and allied reactions. In general terms, it may be stated that the importance of the etiological relationship between the administration of mercury and the toxic arsenical reactions has been overemphasized, although no one can deny that, when the administration of mercury or bismuth has preceded injections of arsphenamin or its substitutes or accompanies these, due care and caution must be exercised in the thorough and routine examination of the urine for albumin and casts, supplemented if necessary by an examination of the blood for urea, total non-protein nitrogen and creatinin and especially by a functional test as advocated by Lorenz and Bleckwerin[14] in order to avoid the grievous mistake of administering full doses of arsphenamin or neoarsphenamin in the presence of damaged kidneys with insufficient elimination.

The administration of the iodids, however, certainly does not appear to be important in relation to this question of toxic reactions by arsphenamin and its substitutes. As shown by Lucke and myself,[15] even very large doses of repeated intravenous injections into rabbits are without injurious effects upon the liver, kidneys and other organs. It is possible and, indeed, probable, that doses large enough to produce ptyalism, eruptions and depressive symptoms of patients may lower local resistance of the gums to mercury and bismuth and by general effects increase susceptibility to the minor reactions of the arsenicals, but there are no grave histological changes produced in the important internal organs as may occur upon the administration of mercury or bismuth.

Hypersensitiveness in Relation to Toxic Reactions by Arsphenamin and Neoarsphenamin.—Finally, one must consider the possibility of natural or acquired hypersensitiveness to arsphenamin, neoarsphenamin and other organic arsenical compounds as a possible cause of untoward reactions following the administration of these to human beings. Undoubtedly, hypersensitiveness to mercury, quinin, procain, iodoform and a few other drugs are known to occur and usually in the form of a natural hypersensitiveness rather than an acquired form due to the use of these drugs.

But before taking up this question, it may be well to define the terms employed, inasmuch as so much confusion exists in the terminology for these phenomena, a more complete discussion of the subject being found in the third edition of my book on "Infection, Immunity and Biologic Therapy." *Allergy* may be defined as a condition of unusual or exaggerated specific susceptibility to a substance which is harmless in similar amounts for the majority of members of the same species. *Anaphylaxis* is a form of allergy characterized by extreme susceptibility to protein agents; *hypersensitiveness* is a form of allergy characterized by extreme susceptibility to non-protein agents. Since the organic arsenical compounds are not protein substances, it is proper to speak of exaggerated susceptibility to them as hypersensitiveness; if, however, it is proven that arsphenamin or its substitutes injected into the blood are capable of uniting with the blood proteins and transforming them into a protein sufficiently "foreign" to the body to sensitize the tissues, then we could speak of these phenomena as being anaphylactic; but this has not been proven, and I believe that "hypersensitiveness" is the proper term to employ in the light of our present knowledge of the subject. *Intolerance*, however, is not the same as hypersensitiveness as defined. Intolerance is due to an accumulation of toxic substances (presumably of pentavalent

compounds of arsenic) in the tissues whereas in hypersensitiveness there may be no evidences of cumulative toxicity.

In the first place does natural hypersensitiveness to arsphenamin and its substitutes (presumably to the arsenic) exist? I have observed immediate reactions of the acute vasoparetic type ("nitritoid crisis") follow the first intravenous injection of 0.45 gm. of neoarsphenamin and personally suspect that these individuals were hypersusceptible to the drug, since the drug itself and method of administration could not be incriminated. Lesne and Dreyfus[16] in 1912 expressed the opinion that some individuals may be naturally hypersusceptible to arsenic, and Zieler[17] at about the same time stated that arsenical dermatitis may be due to a state of hypersensitiveness. All of the phenomena of true allergy in human beings show that the maximum effect is upon the smooth muscle of the body including that of the blood-vessels with the production of vasodilatation followed by cellular and serous exudation. Arsenic likewise produces vasodilatation by some vasculotoxic mechanism and I believe that the acute vasoparetic reaction ("nitritoid crisis") is solely an expression of this vasculotoxic effect of arsenic in individuals who have a natural or acquired hypersusceptibility to the effects of arsenic. The acquired form of this hypersensitiveness due to acquired allergic sensitiveness by reason of intracellular colloidal changes may be readily confused with intolerance due to accumulative toxic effects, but the mechanism is different from that of true hypersensitiveness as defined.

In the second place, may the lower animals be rendered hypersensitive to arsphenamin and its substitutes? All attempts to effect this by intravenous injections of the drugs alone have apparently failed as reported by Auer[18] and others until recently, when success has been claimed by Flandin and Tzanck. In like manner, all similar attempts to render the lower animals hypersensitive by injections of mercury, quinin, belladonna, iodoform, etc., have failed but these failures do not constitute an argument against accepting the possibility of acquired hypersensitiveness in human beings; they simply show that different animals vary in susceptibility and it is very well known that the lesions and symptoms of allergy vary greatly among different species of animals. Likewise, failure to passively transfer drug hypersensitiveness from human beings to the lower animals by injections of serum (passive allergy) does not constitute an argument against accepting the possibility of natural and acquired hypersensitiveness of human beings to drugs, including arsphenamin and its substitutes; the production of passive anaphylaxis of guinea-pigs by injections of the serums of human beings known to be anaphylactic to a protein agent is usually difficult to accomplish, and failure may be due to errors in technic or more likely because the allergic antibody is in or upon the sensitized body cells rather than free in the blood although in this connection mention may be made of the claims of successful passive transfer of allergies to guinea pigs by injections of sera from human beings hypersensitive to iodoform and antipyrin by Bruck[19] and Klausner.[20] Klauder,[21] however, failed to passively transfer to guinea-pigs hypersensitiveness to arsphenamin by injections of serum from a human being who showed all of the classical symptoms (asthma and eczema) of true allergic sensitiveness to both arsphenamin and neoarsphenamin.

Swift[22] claims to have succeeded in producing allergic sensitiveness of guinea-pigs by injecting them with mixtures of guinea-pig serum and arsphenamin, presumably by the production in the test tube of a new protein combination sufficiently "foreign" to sensitize and intoxicate the animals; but as previously stated, no one has yet succeeded in sensitizing any of the lower

animals by intravenous injections of the drugs unless it be Flandin and Tzanck[23] who claim to have successfully sensitized guinea-pigs with intracardiac injections of sulpharsenol in dose of 0.02 gm. They state that the first injection if slowly given produced no symptoms, or, at most, the animals remained motionless, curled up and appeared to be fatigued. A second intracardiac injection, made not less than three days later, and of $\frac{1}{10}$th to $\frac{1}{20}$th the first dose, induced what they regarded as allergic crises in two minutes from which the majority of animals recovered. The symptoms, as described by them, appear to me, however, as being possibly due to "anaphylactoid" phenomena as described by Hanzlik and Karsner[24] and the experiments require confirmation before one may accept them as true allergic reactions.

However, may human beings acquire hypersensitiveness as a result of injections of arsphenamin and its substitutes? The fact that guinea-pigs have not been generally successfully hypersensitized by injections of arsphenamin and neoarsphenamin does not by any means answer the question for human beings, because the latter are apparently more susceptible to sensitization by various substances than the lower animals with the possible exception of the guinea-pig by injections of horse serum. In 1911, Fischer,[25] Hoffmann and Jaffe,[26] Levens[27] and Swift expressed the opinion that human beings may acquire hypersensitiveness to salvarsan and at about the same time Staeubli[28] described a case of apparent acquired hypersensitiveness to sodium cacodylate yielding positive skin reactions, similar cases being reported by Kauffmann[29] and Stauble.[30] More recently Stuart and Maynard[31] and Guy[31a] have described cases of apparently acquired hypersensitiveness to arsphenamin among some patients who suffered with exfoliating dermatitis and describe well-marked skin reactions following the intradermal injection of dilutions of neoarsphenamin and arsphenamin varying from 1:1000 to 1:25,000.

Apparently, however, the reactions were not specific for arsphenamin or neoarsphenamin, that is, patients who developed hypersensitiveness to arsphenamin yielded positive skin reactions likewise to neoarsphenamin. Mook[32] had previously observed negative reactions when solutions of arsphenamin had been applied to abrasions of the skin and Moore and Keidel[33] have likewise observed negative cutaneous tests among some of their cases of dermatitis. Klauder,[34] however, observed positive cutaneous reactions following the application of 2-per cent. solutions of arsphenamin and neoarsphenamin to scratches on the arm of a marked case of apparent hypersensitiveness to these drugs with asthma and localized eczematous dermatitis of the hands and face.

It is difficult to reach a conclusion on this subject of acquired hypersensitiveness to arsphenamin and neoarsphenamin; it may be, as stated by Ullmann,[35] that, in cases suggestive of this state that there is deficient elimination and cumulative toxic effects rather than a true hypersensitiveness but I personally believe that hypersensitiveness may be acquired. Not infrequently patients who have had one or more courses of arsphenamin or neoarsphenamin suddenly or gradually begin to develop immediate or early toxic reactions after the intravenous injection of even small doses of neoarsphenamin; it is true that skin tests among these have been uniformly negative and that the effects may have been due to accumulative toxicity but this is hardly likely when nausea, vomiting, flushing, etc., develop almost immediately after the intravenous injection of 0.2 to 0.3 gm. of neoarsphenamin and when one may be reasonably sure that neither the drug nor method of administration are at fault.

In conclusion, brief mention may be made again of the opinions expressed by Stokes, Keidel and Moore to the effect that arsphenamin dermatitis, while not directly the result of hypersensitiveness to the drug, may be due to a state of anaphylaxis to bacterial or other foreign protein brought to the skin by means of the vasculotoxic effects of the arsenic of the drugs; these theories are discussed with more detail in Chapter LI.

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CHAPTER XLVIII

ADMINISTRATION OF ARSPHENAMIN, NEOARSPHENAMIN AND SULPHARSPHENAMIN IN RELATION TO TOXIC REACTIONS

Errors in the administration of arsphenamin, neoarsphenamin and their substitutes employed in the treatment of syphilis have always been prominently mentioned as the cause of untoward effects and reactions. As I have already discussed in Chapter XLV, this subject is of particular importance in the administration of arsphenamin owing to the importance of physical

and physico-chemical factors greatly influencing the toxicity of this compound, but the methods employed for preparing and administering solutions of neoarsphenamin, sulpharsphenamin and other medicinal organic arsenicals are likewise of considerable practical interest and importance in relation to toxic reactions and therapeutic results.

In order to discuss this subject in a logical manner I shall endeavor to correlate the results of animal experiments with clinical experience and to present the matter in steps corresponding to the order in which the drugs are prepared for administration beginning with their keeping qualities as dispensed, the preparation of solutions, etc., and ending with their injection.

Stability or Keeping Qualities of Arsphenamin, Neoarsphenamin and Sulpharsphenamin. *Deterioration of Arsphenamin, Neoarsphenamin and Sulpharsphenamin Powders Exposed to Air.*—Arsphenamin, neoarsphenamin and sulpharsphenamin are usually dispensed in ampules from which the air has been withdrawn by vacuum production or in which it has been replaced by an oxygen-free gas as nitrogen or carbon dioxid. This precaution is for the purpose of preventing oxidation and other changes which increase the toxicity of the compounds. The physician, therefore, must carefully avoid using cracked ampules and especially those in which the powders have changed to a deep orange or brown color with decreased solubility.

As a matter of fact, however, perfectly dried and freely movable arsphenamin, neoarsphenamin and sulpharsphenamin powders exposed to the air at ordinary room temperature in open ampules do not quickly become toxic or change in trypanocidal or spirocheticidal activity. In 1916, Schamberg, Raiziss and I[1] reported that arsphenamin powder exposed to the air in open ampules from eighteen to forty-one days did not acquire increased toxicity for rats. Since then I have repeatedly tested the toxicity of different brands of arsphenamin powders kept in open ampules for from one hour to twenty-one days at ordinary room temperature and found only occasionally an increase of toxicity of some lots after an exposure of five or more days without any definite decrease in trypanocidal activity. Neoarsphenamin and sulpharsphenamin, however, are much less stable and have usually shown an increase of toxicity after two or three days exposure, although in some instances no change was apparent for ten or more days. It is to be carefully borne in mind, however, that these tests were conducted with rats injected intravenously according to the official method described in Chapter II and the results based upon the largest amounts of the drugs compatible with life, whereas with human beings it is entirely likely that the exposed compounds would prove much more toxic, as indicated by the severe reactions known to have been produced by the accidental administration of the drugs from cracked ampules in which the powders have been exposed for indefinite periods of time.

Not infrequently ampules are opened but the contents not dissolved for an hour or two, and the question arises whether or not it is safe to use arsphenamin, neoarsphenamin or sulpharsphenamin under such conditions. I believe that the danger of oxidation of the dried powders has been very much over-emphasized. Repeated and rigid animal tests have proved to me that these compounds do not acquire an increased toxicity when the powders are exposed to the air for an hour or two and I have repeatedly administered them by intravenous injection without any untoward effects whatsoever. Solutions exposed to the air deteriorate much more rapidly, as will be shortly discussed, but arsphenamin, neoarsphenamin and sulpharsphenamin in dried powder form can be safely used an hour or two after the ampules have been opened. An exposure of this duration does not result in any change in color,

solubility, toxicity or spirocheticidal activity of these compounds and undue haste in the preparation of solutions is unnecessary.

Deterioration of Arsphenamin, Neoarsphenamin and Sulpharsphenamin Powders Sealed in Vacuo.—Probably of even greater practical importance is the question of the keeping qualities of arsphenamin, neoarsphenamin and sulpharsphenamin properly sealed in ampules *in vacuo* or with an oxygen-free gas. Physicians not infrequently employ compounds one or more years old that have been kept in their offices or in pharmacies for long periods of time at ordinary room temperature and the matter of changes in toxicity and therapeutic activity of such compounds becomes one of considerable practical importance.

Roth[2] has reported that 25 to 30 per cent. of different lots of two brands of neoarsphenamin examined in the Hygienic Laboratory three years after the first tests showed changes of deterioration and that changes in solubility of neoarsphenamin may occur as early as forty-eight hours after manufacture. My own experiments with three different brands of neoarsphenamin kept at ordinary room temperature for a period of two years have not shown quite as high percentage of deteriorations as reported by Roth occurring in three years. From 5 to 12 per cent. of ampules, however, have shown changes in color to a deep orange or a change in solubility and a few of these showed a slight increase of toxicity for the rat in that the maximum tolerated dose was reduced from 0.280 to 0.350 gm. per kilo to 0.190 to 0.300 gm. per kilo. In no instance have all the ampules of any single lot shown this change but only a few ampules of certain lots. In other words, it would appear that deterioration may be due to defects in putting up the ampules and especially insufficient vacuum; likewise, in this connection, the question of the dryness of the powder is important and experience has shown tht neoarsphenamin, weighed out and ampuled on humid, rainy days with increased moisture in the air, are apt to deteriorate more rapidly and more frequently than perfectly dry products ampuled on dry, sunny days. The trypanocidal and spirocheticidal activities of the powders in such ampules, however, have shown no greater degree of change than the usual and expected differences, due to experimental error, because the degree of oxidation and other chemical change in the molecules has not usually progressed to the extent of reducing the parasiticidal properties as may occur, however, when solutions are exposed to the air until profound changes have occurred.

Arsphenamin is much more stable. Roth has observed practically no changes in the physical appearance, solubility or toxicity of different brands kept in the Hygienic laboratory for three years and my own experience with different lots from one laboratory kept for as long as seven years has been the same. I have repeatedly determined the toxicity and trypanocidal activities of arsphenamin from one to three and a half years old without detecting any degree of change beyond slight and irregular differences due to experimental error. In a fewer number of spirocheticidal tests employing rabbits infected intratesticularly with *Spirochæta pallida*, no changes were discovered with products four years old.

It is well, however, for physicians, clinics, hospitals and pharmacies to avoid overstocking with these compounds in order that it may be a matter of routine to use fresh rather than aged arsphenamin and neoarsphenamin and especially the latter.

I do not know how sulpharsphenamin compares with arsphenamin and neoarsphenamin in these respects, since this compound has been in use for only about two years. At this writing it may be stated, however, that I have found no evidences of color change, solubility, toxicity and trypanocidal activity of different lots of two brands kept at ordinary room temperature for twelve months.

Deterioration of Preserved Solutions of Arsphenamin (Lowy).—Since arsphenamin is quite a stable substance even in solution, provided all oxygen has been removed, Lowy[3] has advocated the clinical use of prepared solutions in order to facilitate the administration of the drug by those physicians who have neither the time nor the facilities for making up their own solutions.

When properly prepared with a high degree of vacuum to remove oxygen, these solutions can be kept for six months or longer without changes in physical appearance or increased toxicity. But I have observed that they may undergo a gradual reduction in trypanocidal activity and the Hygienic laboratory has placed a *time limit of six months upon them dating from the time of their preparation.* In other words, solutions of arsphenamin prepared according to Lowy's method are perfectly satisfactory for at least six months and serve a very useful purpose for aiding in the administration of the drug by those physicians who inject the compound only occasionally, but after six months changes in toxicity, and more especially, a reduction of therapeutic activity may occur. These solutions do not appear to be more toxic for the rat than freshly prepared solutions and clinically they are just as satisfactory, as indicated by the reports of Stetson[4] and others.

Solutions of arsphenamin (Lowy) should be kept in a cold place; at temperatures higher than 75°F. deterioration is more rapid. Freezing does no harm if the solution is allowed to thaw at a temperature not higher than 70°F.

Menstrum Employed for Preparing Solutions in Relation to Toxic Reactions.—Since the earliest days of arsphenamin therapy, some of the toxic reactions have been ascribed to the water or saline solution employed instead of to the drug. Inasmuch as the ordinary dose of this compound is administered by intravenous injection dissolved in 100 to 200 c.c. or more of water, saline solution, etc., the condition of the menstrum is not an unimportant matter; within recent years, however, the subject has not commanded as much attention as formerly, since neoarsphenamin and sulpharsphenamin administered in concentrated solutions have largely replaced arsphenamin in the treatment of syphilis and thereby reduced the importance of those toxic reactions possibly due to foreign substances and especially organic matter in the menstrum employed.

Microörganisms and Their Products and Other Matter in the Menstrum, in Relation to Toxic Reactions.—When *sterile* water or saline solution is employed no part of the toxic reaction can be ascribed to living bacteria; sterilization may also be counted upon to destroy bacterial toxins, so that one cannot subscribe to the original "Wasserfehler" theory of Wechselmann[5] that the febrile and gastro-intestinal reactions were due to *living* hemolytic bacteria and their toxins in the water or saline employed when the solutions are sterilized just before use.

But sterilization does not remove the possibility of reactions being caused by dead bacteria and various yeasts and molds and their disintegration products that may be present. It is well known that the intravenous injection of as few as 25,000,000 dead colon or typhoid bacilli in the form of a vaccine may elicit a sharp reaction of fever and rigors; these effects are not due to living bacteria and toxins, but rather to the disintegration products of the organisms and the colloidal disturbances which their injection produces in the form of the so-called non-specific protein reaction. Now *stale* water, distilled or otherwise, frequently and indeed usually, contains a surprisingly large number of bacteria and yeasts unless due precautions are taken to prevent contamination after distillation. Distillation kills bacteria, destroys their toxins and largely leaves behind the products of their disintegration; when such water is carefully protected against subsequent contamination, the possibility of "protein" toxic reactions is greatly reduced but not infrequently solutions of arsphenamin and its substitutes are prepared with stale distilled water in which precautions against contamination are very inadequate as, for example, the use of the different commercial brands of

bottled distilled water. It is true that such water can be and usually is, sterilized before use; this, however, only kills the organisms but does not remove them and I believe that the intravenous injection of a large volume of such water as 80 to 300 c.c., may elicit a reaction of fever, nausea, rigors, etc., in exactly the same manner as the intravenous injection of a sterile bacterial vaccine.

Yakimoff and Yakimoff[6] have stated that salvarsan injected into animals along with various dead bacteria is more toxic than salvarsan alone; that is to say, when such bacteria are present, a smaller dose of salvarsan will suffice to kill the animal than when no bacteria are present. McIntosh, Fildes and Dearden[7] have shown in a clear and convincing manner experimentally and clinically that bacteria-free saline solution is non-toxic, whereas the addition of different varieties of bacteria to non-toxic saline before sterilization. renders it toxic due to the presence of the dead bacterial bodies themselves and not to endotoxins derived from them. Matzenauer,[8] however, claims that water containing as many as 1,700,000 germs per cubic centimeter did not increase the toxicity of salvarsan and believes that toxic reactions are due primarily to the drug itself. In 1916, Schamberg, Raiziss and I[9] showed that arsphenamin dissolved in freshly distilled water was less toxic for rats than solutions in stale distilled or non-distilled water.

Therefore, one of the chief purposes of using *freshly* distilled water for preparing solutions of arsphenamin and its substitutes for intravenous injection, is to remove dead microörganisms and their products; Ehrlich always insisted upon this with justice and reason. *Freshly* sterilized tap water containing but few bacteria is much to be preferred to a *stale* sterilized distilled water and it is surprising how few bacteria are usually found in fresh tap water. Many physicians have reported that they have employed fresh sterilized tap or spring water with as much success as sterilized distilled water and this is readily understood, provided the raw water is not unusually rich in inorganic salts or organic matter.

It is true that this subject is not as important in relation to the injection of concentrated (10 to 20 c.c.) solutions of neoarsphenamin or sulpharsphenamin as it is to the intravenous injection of arsphenamin dissolved in 80 to 300 c.c. of water or saline solution; but the intravenous injection of even 10 to 20 c.c. of stale water containing dead bacteria and yeasts may conceivably produce reactions. At any rate the matter of the purity of the water or saline solution from the standpoint of bacterial proteins and other organic matter is not without practical importance, and here it may be stated that solutions should be prepared only with water or saline that is not only sterile but free or nearly so of dead microörganisms and their products and of other organic matter; therefore, only freshly distilled water or distilled water hermetically or otherwise sealed against subsequent contamination should be used for the intravenous injection of arsphenamin and its substitutes.

Inorganic Impurities in Water or Saline, in Relation to Toxic Reactions.— Distillation of water also removes a large part of inorganic salts and this may be an added advantage in those districts in which the water is unusually rich in various salts of calcium, magnesium, etc. Gonder, at the request of Ehrlich, conducted experiments on the toxicity of salvarsan solutions to which small amounts of calcium and magnesium salts commonly found in tap water, were added and states these increased the toxicity of the drug for mice and other animals. Emery believed that contamination of water with copper, lead or salicylates from the distilling apparatus increased the toxicity of salvarsan solutions. Matzenauer believed that alkali dissolved out of the glass container may be a factor in the production of reactions, etc.

These observations led Ehrlich and others to advise the use of water freshly distilled from glass apparatus and various kinds have been devised to enable physicians to distil water in their offices. Personally I have never

been convinced that all-glass apparatus was necessary and, so far as my animal tests and clinical experience are concerned, water distilled from tin-lined apparatus has always been just as satisfactory, provided the boiling and condensing apparatus is occasionally cleaned to remove encrusted salts. In my opinion the primary purpose of using freshly distilled water is to avoid an excess of organic matter and especially the products of dead organisms; the usual amounts of inorganic salts present in water are of much less importance.

Sterility of Arsphenamin, Neoarsphenamin and Sulpharsphenamin.—In connection with this subject of bacterial contamination in relation to toxic reactions, the question may arise regarding the sterility of the drugs themselves. A few years ago I cultured aërobically and anaërobically 100 different lots of arsphenamin and 100 different lots of neoarsphenamin and also made mouse inoculation tests for tetanus spores. All of the arsphenamin cultures proved sterile; ten lots of neoarsphenamin showed the presence of bacteria, eight of these cultures being *B. subtilis* and two a white staphylococcus. All mouse tests for tetanus proved negative. Arsphenamin, therefore, has proved more bactericidal than neoarsphenamin; this is true not only of the dried powder but of the solutions as well, as will be shortly discussed.

These results, however, warrant the conclusion that when arsphenamin and neoarsphenamin are tubed in a careful and aseptic manner bacterial contamination is quite uncommon and in this connection it may be stated that no cases of bacterial infection have yet been ascribed to the injection of these compounds; of course, this statement does not refer to local skin infections due to faulty technic in injection. Since sulpharsphenamin is frequently administered by subcutaneous or intramuscular injection, the question of bacterial contamination is of greater practical importance in relation to the local reactions produced; up to the present time, however, my cultures of twenty-two different lots of the sulpharsphenamines prepared in two different laboratories have proved sterile in all except two; apparently this product is less bactericidal than arsphenamin. At any rate, it does not appear that either local or general bacterial infections have been ascribed or traced to the drugs themselves and it is certain that they do not contain enough living bacteria to produce the protein reactions.

Bactericidal and Bacteriostatic Activity of Solutions of Arsphenamin and Neoarsphenamin.—And now the question may also arise regarding the bactericidal properties of solutions of arsphenamin and its substitutes, not so much in relation to their use in the treatment of bacterial infections considered in Chapter VII, as in relation to the autosterilization of solutions before administration.

With the assistance of Miss Mary Trist, I have studied this question with much interest employing the Hygienic laboratory phenol coefficient method and my bacteriostatic test previously described.[10] Arsphenamin dissolved in cold water in the usual proportions of 0.6 gm. to 80 to 150 c.c. followed by neutralization, is not appreciably bactericidal in 15 minutes, but if boiling hot water is employed a considerable degree of bactericidal activity for staphylococci is apparent, largely due to the heat in addition to the feeble activities of the alkali and drug itself. In other words, *the use of very hot water for the preparation of solutions of arsphenamin has the distinct advantage of greatly increasing the chances of infecting a sterile solution in case the glassware or water had become accidentally contaminated.*

One per cent. solutions of neoarsphenamin and sulpharsphenamin were not bactericidal but concentrated solutions like 0.6 gm. dissolved in 6 c.c. of water were frequently completely bactericidal in 5 minutes, almost always in 15 to 30 minutes and may be counted upon to bring about a con-

siderable degree of bactericidal activity in case the water or glassware has become contaminated.

Water versus Saline Solution in Relation to Toxic Reactions.—The question has frequently been raised whether saline solution alone was responsible for mild febrile reactions or "salt fever," since Marschalko[11] in 1911 stated that the percentage f sodium chlorid present in the saline solution was a matter of great importance. Bingel[12] found that fever was elicited in man by subcutaneous and intravenous injections of normal saline solution, but that the result was not influenced by alterations in the concentration of sodium chlorid. Wechselmann has stated that the occurrence of fever was largely diminished by the use of Ringer's solutions, but McIntosh, Fildes and Dearden[13] found that toxic reactions were very little less frequent after intravenous injections of Ringer's solution than after saline and that alterations in the concentration of sodium chlorid did not affect toxic reactions. Indeed, quite recently, Wiesenack[14] has advocated the use of hypertonic saline solution for the administration of arsphenamin, stating that it reduces the incidence of reactions.

In numerous toxicity and trypanocidal tests employing rats, I have never been able to observe that solutions of arsphenamin and neoarsphenamin in water varied from duplicate solutions in saline solution, although it is to be borne in mind that tests of this kind do not disclose the incidence of minor reactions as fever and rigors which may be elicited in human beings. White rats can stand intravenous injections of enormous amounts of distilled or tap water and physiological saline solution without the production of jaundice, hemoglobinuria or other discoverable evidences of toxic reactions; as much as 50 c.c. per kilo corresponding to 3000 c.c. per 60 kilos or the weight of an average sized adult human being have been injected without influence upon the lives of these animals. But as just stated, the intravenous injection of water or saline solution may produce reactions in man which are not discernible in the rat, although it does not appear that physiological saline solution is any more toxic than an equal amount of distilled water per kilogram of weight. The intravenous injection of either usually produces a mild leukocytosis which may be accompanied by slight fever if large amounts are administered. In rabbits, the intravenous injection of 0.5 c.c. of water per kilo generally produces a slight leukocytosis with an increase of the polymorphonuclear leukocytes and an increased rectal temperature of 1° or 2°F. within two to four hours after injection, and, curiously, in numerous experiments, I have observed that the injection of freshly distilled water produces slightly greater reactions than tap water.

While saline solution does not appear to increase the incidence of toxic reactions, the question now arises whether it reduces them. I believe it may be stated in a broad and general manner that, when arsphenamin, neoarsphenamin or sulpharsphenamin are injected intravenously dissolved in 100 c.c. or more of water or saline solution, the latter may reduce the incidence of reactions and should be employed because intravascular hemolysis is prevented or reduced. As discussed more fully in Chapter XLV neoarsphenamin and sulpharsphenamin are not hemolytic but arsphenamin is hemolytic. Furthermore, when the usual dose of neoarsphenamin or sulpharsphenamin is dissolved in 10 to 15 c.c. of water the solutions are not hemolytic, but solutions of neoarsphenamin or sulpharsphenamin in 30 c.c. or more of water are not isotonic and may produce hemolysis. Solutions of the monosodium and disodium salts of arsphenamin in both water and saline solution are hemolytic, but the latter are much less so than the former. *Therefore, when neoarsphenamin and sulpharsphenamin are administered in concentrated solutions, as they may well be, sterile freshly distilled water may be employed, but when these and arsphenamin are administered in dilute solutions it is better to use a sterile* 0.5*-per cent. saline solution prepared of chemically pure sodium chlorid and freshly distilled water.*

Glucose Solutions in Relation to Toxic Reactions and Therapeutic Activity. A 4.5- to 4.7-per cent. solution of chemically pure glucose in distilled water

is isotonic and Fleig[15] states that solutions of arsphenamins in 4.5-per cent. glucose are less toxic for both rabbit and man by intravenous injection than solutions in water. Isotonic solutions of lactose (9.2- to 9.5-per cent.) likewise reduced toxicity to some extent, but not as much so as 4.5-per cent. solutions of glucose. Hypertonic glucose solutions (10-per cent.) also decreased toxicity, but best results were observed with the isotonic solutions.

The results of my own experiments employing rats have yielded confirmation to these observations. In the first place, chemically pure glucose is but very slightly toxic. I have injected rats intravenously with doses as high as 25 grams per kilo of body weight without fatal results and this dose corresponds to 1500 grams for an average adult of about 132 pounds.

Solutions of the disodium salt of arsphenamin in water, killing rats within forty-eight hours after intravenous injection is dose of 0.140 gm. per kilo of weight, have been borne indefinitely in doses as high as 0.220 gm. per kilo administered in 4.5- to 5-per cent. solutions of glucose in water. Numerous experiments have yielded similar results in that the use of glucose solution has reduced the toxicity of arsphenamin for these animals. Furthermore, my trypanocidal tests have shown that the therapeutic activity of arsphenamin is not altered (increased or decreased) in isotonic glucose solution and from the standpoint of animal tests it would appear advisable to employ sterile 4.5- to 5-per cent. solutions of chemically pure glucose in distilled water (sterilized in an Arnold and not autoclaved) for the administration of arsphenamin, although I am not convinced that the incidence of reactions in human beings is materially lessened as compared with solutions in plain isotonic saline.

I am quite sure that there is no advantage in using glucose solution for the intravenous administration of concentrated solutions of neoarsphenamin and sulpharsphenamin, although it is stated that solutions of the latter, as well as of soluble sodium and potassium tartrobismuthate in glucose solutions, are less painful when injected subcutaneously or intramuscularly. Duhot[16] employs a 50-per cent. solution of glucose for intravenous injections and states that it prevents the precipitation of arsphenamin by the plasma and thereby reduces its toxicity. Cheinisse[17] likewise employs hypertonic solutions (25- to 50-per cent.) for the administration of arsphenamin to hypersensitive patients, and states that this plan enabled him to continue the treatment of five individuals who previously had been totally unable to tolerate the drug. Pranter[18] employs 10- to 20-per cent. solutions; Steinberg[19] administers 0.1 to 0.2 gm. doses of both arsphenamin and neoarsphenamin in 30 c.c. of 30-per cent. solutions of Merck's glucose and states that not only are the toxic reactions reduced but that spirocheticidal effects are increased. As stated above, I found that 5-per cent. solutions had no effect upon trypanocidal activity, but since glucose reduces toxicity by lowering the rate and degree of reduction of arsphenamin and neoarsphenamin by oxidation, I am inclined to think that Steinberg is in error and that the effect of large amounts of glucose would be to reduce the toxicity of the drugs for the spirochetes, that is, reduce therapeutic activity, in the same manner as it reduces toxicity for body cells.

Gelatin Solutions in Relation to Toxic Reactions and Therapeutic Activity.—In Chapter XLV I have already discussed the work of Oliver and his associates upon the influence of gelatin in reducing the toxicity of arsphenamin presumably by protecting the corpuscles against agglutination and hemolysis.

In my experiments it was found that the maximum tolerated dose of gelatin, dissolved in sterile distilled water but *not autoclaved*, was approximately 6 to 7 grams per kilo; solutions *autoclaved* for 20 minutes at 15 pounds pressure were less toxic, the maximum tolerated dose being more than 10 grams per kilo. From the standpoint of toxicity it would appear, therefore, quite safe to inject human beings with 0.5- to 2-per cent. solutions intravenously.

Unquestionably solutions of disodium arsphenamin in 1- to 2-per cent. solutions of gelatin in sterile distilled water are sometimes slightly less toxic for rats by intravenous injection than solutions in plain water and in my experiments solutions of arsphenamin in autoclaved 2-per cent. solutions of gelatin were less toxic than in unheated solutions of

gelatin. But gelatin solutions of arsphenamin, as well as of neoarsphenamin, have generally (but not always) been slightly less trypanocidal and spirocheticidal in my tests than solutions in water and I believe this constitutes an objection to the use of gelatin solutions for the administration of arsphenamin in the treatment of syphilis.

Furthermore, I am not at all sure that 0.5- to 2-per cent. gelatin solution reduces the incidence of toxic reactions in human beings; indeed, it is my impression that the intravenous injection of 100 to 150 c.c. of these solutions has frequently yielded reactions of slight fever, chilliness and malaise in from one to three hours after injection that were caused by the gelatin rather than the drug.

Influence of the Temperature of the Water or Saline Solution Employed upon Toxic Reactions.—The possible influence of the temperature of the menstrum employed for the preparation of solutions of arsphenamin is of considerable practical importance, because some preparations are not freely soluble in cold water, rendering necessary the use of hot water; indeed, it is not an uncommon practice to add the drug to water boiling freely over a flame and I have myself usually employed this method. Since neoarsphenamin and sulpharsphenamin are freely soluble in cold water, there is no necessity for using hot water although the influence of the latter upon toxicity is not without interest since some physicians employ hot water.

Lake[20] has reported that two different brands of arsphenamin dissolved in cold water and alkalinized in the usual manner killed 17 per cent. of rats within forty-eight hours in dose of 0.100 gm. per kilo by intravenous injection; solutions prepared with boiling hot water gave identical results, so that the use of boiling hot water for preparing solutions had no influence upon toxicity. When, however, the cold and hot solutions were placed in a boiling water bath for five to sixteen minutes, cooled and alkalinized, the toxicity was found to be increased, since 65 to 70 per cent. of the animals succumbed. Hunt,[21] however, found that solutions prepared with cold water and alkalinized followed by heating to 40° to 60°C. for a few minutes rendered the drug less toxic. The results of my experiments in this field, which have been quite numerous with different brands of arsphenamin and neoarsphenamin and employing rats injected intravenously, may be summarized as follows: (*a*) Arsphenamin dissolved in water at 60°–70°C. and boiling hot water, followed by cooling and alkalinization, was not any more toxic and possessed the same trypanocidal activity as solutions prepared in cold water. (*b*) Solutions prepared in hot or cold water followed by boiling for more than 5 minutes, cooling and alkalinization were usually rendered more toxic. (*c*) Solutions prepared in hot or cold water, alkalinized and then boiled were rendered still more toxic, that is to say, alkalinized solutions are rendered more toxic by heating than acid solutions. (*d*) Neoarsphenamin and sulpharsphenamin dissolved in boiling hot water were usually (not always) more toxic than solutions in cold water. (*e*) Solutions of neoarsphenamin and sulpharsphenamin boiled for five minutes or longer were usually rendered more toxic and less trypanocidal.

From the practical standpoint, therefore, this subject may be summarized as follows: (*a*) Arsphenamin may be dissolved in boiling hot water without any increase in toxicity or loss in therapeutic activity. (*b*) As soon as the solution is made, which should not require more than a few minutes, it should be allowed to cool. (*c*) After alkalinization the solution may be warmed or maintained at body heat without increase of toxicity. (*d*) Neoarsphenamin and sulpharsphenamin are least toxic when dissolved in cold water and deteriorate more rapidly than arsphenamin at high temperatures. To the best of my knowledge, solutions of arsphenamin prepared with hot water are not any more toxic for human beings than solutions prepared in cold water; indeed, the reverse is probably true, since arsphenamins difficulty soluble in cold water are chemically purer than those freely soluble in cold water. But solutions of neoarsphenamin in hot water may be more toxic than solutions in cold water; I know of one instance in which three cases of dermatitis were regarded as probably due to the administration of neoarsphenamin dissolved in hot water. However, since neoarsphenamin and sulpharsphenamin are freely soluble in cold water, there is no advantage in using hot water.

Use of Alcohol in the Preparation of Solutions of Arsphenamin, in Relation to Toxic Reactions.—One difficulty in preparing solutions of those arsphenamins not freely soluble in cold water is the formation of gelatinous masses which require an undue amount of shaking to effect complete solution. By placing the powder in a dry cylinder and thoroughly moistening with 1 or 2 c.c. of pure ethyl alcohol, quick solution is usually accomplished with cold or warm water, or saline solution with much less shaking. Lake has found in the Hygienic Laboratory that solutions prepared in this manner are sometimes appreciably less toxic than solutions made up in the shortest possible time with hot water. My own experience has been similar; toxicity for rats by intravenous injection has never been increased and in some instances has been somewhat less. Trypanocidal activity has never been altered. I have also administered arsphenamin prepared in this manner to human beings with no increase of toxic manifestations and regard the method as quite satisfactory for effecting solutions of those arsphenamins not freely soluble in cold water. I cannot say, however, that the results clinically are any better than solutions prepared with boiling hot water, as described in Chapter LVIII, but there is no danger of rendering the solutions more toxic by undue or prolonged heating and the method can be recommended for clinical use. The alcohol appears to increase the solubility of arsphenamin, not by dissolving it, but by penetrating throughout and moistening the dry powder. When water is added and the container quickly shaken, no gelatinous masses form as in the case when water alone is employed, these masses being the principal cause of delay in making solutions.

Solubility of Arsphenamin and Neoarsphenamin in Relation to Toxicity.—As previously stated, the less soluble brands of arsphenamin are not any more toxic than those readily soluble in cold water if simple precautions are taken against too much shaking of the solutions and overheating. From the chemical standpoint these arsphenamins are regarded as being chemically purer than those readily soluble in cold water.

But this is not true of neoarsphenamin. Solutions of this drug in water which are cloudy or opalescent are almost invariably more toxic for the lower animals and man than perfectly clear solutions. Not even filtration of the former through sterile paper or cotton removes the toxic factors and it is extremely important clinically to guard against the intravenous injection of either neoarsphenamin or sulpharsphenamin unless the solutions are readily made without undue shaking and are crystal clear. As previously stated, neoarsphenamin tends to deteriorate in the ampules and one sign of this change is decreased solubility. Roth[22] has drawn particular attention to the increased toxicity of incompletely soluble neoarsphenamin for rats due, probably, more to the physical state of the product itself than to excessive shaking; I have been able to confirm his observations and, in addition, have received numerous reports from physicians of severe reactions following the intravenous injection of cloudy solutions of neoarsphenamin which were rendered almost clear by filtration. Roth states that incompletely or slowly soluble products are dangerous, even if filtered. In 1918 to 1920 these products were much more commonly met with than at present; manufacturers have been able to perfect their products in this regard, but the change may occur after the product reaches the physician as a result of deterioration and due care must be exercised against their intravenous administration.

Concentration of Solutions of Arsphenamin and Neoarsphenamin in Relation to Toxic Reactions.—The concentration of solutions of arsphenamin bears an important relation to toxicity for both man and the lower animals; the concentration of solutions of neoarsphenamin and sulpharsphenamin is

much less important. The reason for this is ascribed to the importance of physical factors in relation to the toxic effects of solutions of arsphenamin, such as the reaction, viscosity, etc., as influencing the occurrence or physicochemical reactions like hemagglutination, hemolysis, precipitation of plasma proteins and of drug, etc., as solutions come in contact with the blood.

The subject of concentration of solutions is, therefore, intimately related to the rate of injection, because the amount of drug mixed with the blood in the same length of time may be altered by either factor. But when solutions of arsphenamin of different concentrations are rapidly injected, it has been shown experimentally that the higher concentrations may be more toxic than dilute solutions.

In 1916, Schamberg, Raiziss and I showed that alkalinized solutions of arsphenamin in solutions as concentrated as 0.060 gm. in 2 c.c. of water per kilo of weight, corresponding to 0.6 gm. per 20 c.c., were not any more toxic for rabbits by intravenous injection than dilute solutions, but the injections were given slowly with a gauge No. 26 needle, about three to five minutes being consumed in the administration. Roth has observed that 1-per cent. acid solutions of arsphenamin were approximately twice as toxic for rats as 0.5-per cent. solutions when both were injected at the rate of 1 c.c. in two minutes. Experiments with alkaline solutions administered at the same rate showed that toxicity was but slightly greater as the concentration was increased from 0.5-per cent. (0.6 gm. in 120 c.c. water) to 2-per cent. (0.6 gm. in 30 c.c. water). Fleig, in 1914, also showed that concentrated solutions of arsphenamin sometimes increased the toxicity of the compound and Ehrlich still earlier observed the same effects.

Lake states that a study of the extensive data available in the Hygienic Laboratory, on the clinical use of arsphenamin, showed that reactions seldom occurred when the dilute solution was used, even when the rate of injection was fairly rapid, and that, conversely, even a fairly concentrated solution seldom caused trouble when the rate of administration was slow. The reports showed, however, that it was in clinics where concentrated solutions were used, that most reactions occurred. These observations induced Dr. McCoy, Director of the Hygienic Laboratory, to issue a warning against the use of too concentrated solutions,[23] a concentration not greater than 0.5-per cent. being advised (corresponds to 0.6 gm. in 120 c.c. of water or 20 c.c. for each 0.1 gm.) with the caution that due attention be paid to the rate of injection to guard against too rapid intravenous administration. On the other hand, Nelken[24] considers that concentrated solutions given rapidly are as safe as the more dilute solutions given slowly, and Pusey[25] states that, while it is true that animal experiments have shown arsphenamin to be less toxic when well diluted, the necessity for highly dilute solutions arises only when arsphenamin is unusually toxic.

The influence of concentration is, therefore, far greater with acid solutions of arsphenamin than with those properly alkalinized. Two deaths from arsphenamin brought to my attention were due to the physicians administering 0.4 to 0.6 gm. in 15 to 20 c.c. of water by syringe injection in mistake for sulpharsphenamin. On the other hand, I know of physicians who have injected dilute acid solutions (0.6 gm. in 200 to 300 c.c. of water) without serious reactions; I am sure that only the factor of dilution in these instances averted serious and even fatal reactions.

With properly alkalinized solutions of good arsphenamin, however, the difference in toxicity between 2- and 0.5-per cent. solutions is usually slight or not discernible at all in both the lower animals and man providing that the injections are given *slowly*. I commonly administer 0.4 gm. of disodium arsphenamin in a total of 25 c.c. in a syringe fitted with a No. 22 needle and requiring at least two minutes by the watch with no more reaction than 0.4 to 0.6 gm. in 80 to 120 c.c. by gravity injection. However, if any solu-

tion accidentally infiltrates the perivascular tissues, the concentrated solution produces more reaction than the dilute solution and, since it is difficult to control oneself to give the injection slowly by syringe, I believe it is better routine practice to follow the plan of administration of dilute solutions by gravity injection.

With neoarsphenamin and sulpharsphenamin, however, the influence of concentration and rate of injection are of much less importance, as previously stated. Rats injected intravenously with either compound at the rate of one cubic centimeter per two minutes bear 4-per cent. solutions (corresponding to 0.9 gm. in 22.5 c.c.), as well as 1-per cent. solutions, and the same is true of human beings, even when the concentrated solutions are given by a syringe. It is my practice to administer both compounds by intravenous injection dissolved in not more than 10 to 15 c.c. of water. Indeed, abundant clinical experience has shown that 0.6 and even 0.9 gm. of neoarsphenamin dissolved in 2 c.c. of water (30- to 45-per cent. concentrations) may be injected with a syringe with perfect safety, although a small needle (about gauge No. 22) is advisable to guard against too rapid injection. It is clearly established, therefore, that neoarsphenamin and sulpharsphenamin may be safely administered in concentrated solutions and that dilute solutions injected by gravity are unnecessary.

Influence of Shaking Solutions of Arsphenamin and Neoarsphenamin, in Relation to Toxicity and Therapeutic Activity.—Oxidation of solutions of arsphenamin and neoarsphenamin produces aminohydroxyphenylarsenoxid ("arsenoxid"), which Ehrlich and Hata have shown increases the toxicity of the solutions; for this reason Ehrlich and his collaborators have always warned against unnecessary and excessive shaking of solutions and have advised their immediate injection.

Within recent years more definite experimental data have become available on the influence of shaking solutions of arsphenamin and neoarsphenamin in the air and much of it possesses direct clinical interest and application.

Roth,[26] who was first to study this problem, showed that shaking alkalinized 2-per cent. solutions of arsphenamin for one to ten minutes in cylinders, in contact with an equal volume of air, at the rate of approximately 230 excursions per minute, produced color changes in the solutions and increased their toxicity for rats about 60 per cent. However, this is not of great practical interest, because it is seldom necessary to shake solutions of arsphenamin after the addition of the alkali for more than a few seconds, although Roth states that he has worked with some samples which, upon the addition of normal sodium hydroxid to their watery solutions, formed rather dense precipitates that required considerable shaking, to produce clear solutions.

Of far more practical interest is the effect of shaking acid solutions of arsphenamin, that is, the amount of shaking required to dissolve the powder in water or saline solution. According to Voegtlin and Smith,[27] arsphenamin is exceedingly stable toward atmospheric oxygen, the addition of alkali leading to a rapid increase in the rate of oxidation roughly inversely proportionate to the hydroxylion concentration. Hunt[28] also states that aeration of acid solutions of arsphenamin for from two to four hours did not result in increased toxicity for rats in his experiments. But in the experiments of Schamberg, Raiziss and myself[29] with five different brands or arpshenamin it was found that shaking 2 per cent. acid solutions for one and ten minutes followed by alkalinization sometimes resulted in an increased toxicity for rats injected at the uniform rate of 1 c.c. per two minutes. The increase in toxicity, however, was not quite as great in some experiments as observed when the alkalinized solutions were shaken.

Neoarsphenamin undergoes a similar but even more marked change when solutions are shaken in air. According to Roth, shaking 4-per cent. solutions for ten minutes may increase the toxicity four-fold even when the solution has undergone little or no color change. Schamberg, Raiziss and I have observed similar results with the products of three different laboratories.

Roth has also shown that the color and toxicity changes were due to aeration, since shaking alone in the absence of air produced no changes. Schamberg, Raiziss and I found that the oxygen requirements of shaken solutions of both arsphenamin and neoarsphenamin

were decreased, indicating that oxidation had occurred. With more prolonged shaking it is probable that oxidation would have been more marked accompanied by a decrease in toxicity as "arsenoxid" is oxidized with the formation of m-amino-p-hydroxy-phenylarsonic acid, as apparently occurs when solutions of arsphenamin or neoarsphenamin are allowed to stand for long periods of time in contact with air.

Schamberg, Raiziss and I also determined the effect of shaking solutions of arsphenamin and neoarsphenamin upon trypanocidal activity, that is to say, upon their toxicity for parasites. Rats were employed infected with *T. equiperdum*, the drugs being administered twenty-four hours later by intravenous injection, as described in Chapter X. Voegtlin and Smith[30] state that the trypanocidal activity of arsphenamin is due to the formation of "oxids" and that the administration of preformed "oxid" in the form of "arsenoxid" exerts a quicker and greater trypanocidal effect than injections of arsphenamin itself. Some confirmation of this hypothesis is given by the results of our shaking experiments. Not infrequently the solutions after shaking one minute were more trypanocidal than unshaken solutions, although shaking for ten minutes was sometimes followed by a decrease in trypanocidal activity, probably the result of further oxidation to pentavalent compounds. Nothing can be stated, however, regarding the influence of shaking solutions of arsphenamin and neoarsphenamin upon toxic reactions in human subjects, although there is no reason why the results observed with rats may not apply except that the effects in human beings may not be discernible because the doses administered per weight in the treatment of syphilis are so much smaller than those given rats in the above mentioned experiments. As a matter of fact, additional experiments which I have conducted with acid and alkalinized solutions of arsphenamin and solutions of neoarsphenamin in exactly the same manner except that the dose of arsphenamin was 0.010 gm. per kilo (corresponding to the usual 0.6 gm. dose for the human being) and neoarsphenamin in dose of 0.015 gm. per kilo (corresponding to the usual 0.9 gm. dose) had absolutely no toxic effects. Doubtless the same changes occurred, but the dose was so small that toxic effects were not apparent and I believe the same occurs in the majority of human subjects. From the practical standpoint, therefore, in relation to the treatment of syphilis, the subject may be summarized as follows: (*a*) An undue amount of shaking of solutions of arsphenamin in water may increase toxicity to a slight extent; therefore, with the less soluble arsphenamins it is better to use hot water or moisten the powder with 2 c.c. of alcohol followed by the use of cold water, as previously described. (*b*) After alkalinization the solution should not be shaken more than the few seconds required for dissolving the precipitate. (*c*) Solutions of neoarsphenamin should not be shaken for more than the few seconds generally required. If any neoarsphenamin requires a minute or more of vigorous shaking for effecting solution, it should not be used as the injection of such compounds is likely to produce toxic reactions and especially if the solutions are not quite clear.

Similar experiments with sulpharsphenamin have not been reported, but I believe from a few experiments of my own that this compoud behaves in much the same manner as neoarsphenamin when solutions in water are vigorously shaken in contact with air.

Influence of Exposure of Solutions of Arsphenamin and Neoarsphenamin to the Air in Relation to Toxicity.—Naturally solutions of arsphenamin, neoarsphenamin and sulpharsphenamin allowed to stand exposed to the air may undergo oxidation and coincident changes similar to those just described as a result of shaking in air. But the rapidity and degree of change are not nearly so marked and a great deal that has been spoken and written about the dangers of allowing solutions of these arsenicals to stand before injection needs revision. Ehrlich and his collaborators laid great stress upon the necessity of injecting solutions immediately after their preparation, but we now know that undue haste is not required and that, indeed, in the case of solutions of arsphenamin, it is well to deliberately permit the solutions to stand for ten to twenty minutes in cylinders with the minimum exposure before injection since this results in a decrease of toxicity probably by a re-adjustment of physical factors.

In 1916, Schamberg, Raiziss and I showed that neutral solutions of arsphenamin may be kept in cylinders exposed to the air for four hours and usually for forty-eight hours before there occurred a demonstrable increase of toxicity for rabbits. When cylinders were filled to the stopper in order to exclude the air, toxicity was not increased for many days, even over periods of two to three weeks. On the other hand, in solutions kept in Erlenmeyer flasks and consequently with a greater surface exposed to the air, color changes and

increased toxicity occurred more rapidly than in solutions in cylinders with a much smaller surface of exposure.

Different lots of arsphenamin, however, behaved differently in these experiments, due no doubt to variations in the oxidizability of solutions or to variation in changes of physical factors concerned in toxic reactions. Sometimes solutions exposed for five days or longer, with marked color change and the formation of precipitates requiring an additional drop or two of alkali to effect their solution before intravenous injection, were less toxic than solutions standing but two or three hours—indeed, in some instances, less toxic than solutions injected immediately; these parodoxical results may have been due to oxidation to the point when the less toxic and less trypanocidal pentavalent compounds were produced.

Acid solutions of arsphenamin are more stable when exposed to the air than alkalinized solutions and undergo color changes and increased toxicity more slowly; neoarsphenamin and nepharsphenamin are quite susceptible to change. Color changes characterized by a gradual conversion of the usual light canary yellow solutions to greenish-yellow, green and finally blackish-green solutions are probably not due to the formation of "arsenoxid," which occurs as a white substance, but rather to oxidation of the amino-groups. Therefore, asolution may become toxic withuot coincident color change, as Roth observed with the solutions of some lots of deteriorated neoarsphenamins, but, as a general rule, a change in color to dark greenish-yellow indicates an increase of toxicity; clinically when a preserved solution of arsphenamin (Lowy) is found to have undergone such a change, it should not be employed.

Roth[31] has confirmed our observations that solutions of disodium arsphenamin may be kept in partly filled cylinders for at least five hours before an increase of toxicity for rats could be observed and more recently has pointed out that solutions allowed to stand for ten to twenty minutes are frequently less toxic for these animals than solutions injected at once. Hunt has likewise observed that solutions of some lots of arsphenamin kept in cylinders tend to lose in toxicity either within ten minutes after preparation or sometime within four hours, although different preparations varied greatly in this respect. Very probably this decrease in toxicity of arsphenamin solutions kept in cylinders with slight exposure to air is due to a decrease in the toxic physical factors without the coincident production of sufficient oxidized compounds to increase toxicity.

During the past year Dr. Schamberg and I, with the assistance of Miss Rule, have re-studied the influence of standing upon the toxicity of solutions of disodium arsphenamin in water and 5-per cent. glucose solutions and upon the toxicity of solutions of neoarsphenamin in water and glucose solution, the solutions being kept at ordinary room temperature in *Erlenmeyer flasks* with a large surface of exposure to the air. The maximum tolerated dose of each per kilo of weight was determined for rats of regulation weight by intravenous injection, with the following results:

	Disodium Arsphenamin in Water		Disodium Arsphenamin in Glucose Solution	
	Color	M.T.D.	Color	M.T.D.
Injected at once	No change	0.190	No change	0.200
Injected after 1 hour	No change	0.190	No change	0.200
Injected after 2 hours	No change	0.178	No change	0.200
Injected after 4 hours	No change	0.140	No change	0.200
Injected after 24 hours	Greenish-brown	0.120	Brownish	0.200
Injected after 72 hours	Black-green; ppt.	0.100	Brownish; no ppt.	0.160

	Neoarsphenamin in Water		Neoarsphenamin in Glucose Solution	
	Color	M.T.D.	Color	M.T.D.
Injected at once	No change	0.400	No change	0.400
Injected after 1 hour	No change	0.400	No change	0.400
Injected after 2 hours	No change	0.250	No change	0.250
Injected after 4 hours	No change	0.100	No change	0.100
Injected after 24 hours	Light green	0.100	Light-green	0.100
Injected after 72 hours	Brown-green	0.050	Brown-green	0.050

It will be observed that neoarsphenamin becomes toxic upon standing freely exposed to the air more rapidly than solutions of alkalinized arsphenamin and that solutions of disodium arsphenamin in 5-per cent. glucose undergo color changes and increased toxicity much more slowly than solutions in water; on the other hand, glucose solutions of neoarsphenamin behaved in very much the same manner as solutions in plain sterile distilled water.

From these experiments it would appear that solutions of disodium arsphenamin in water do not undergo appreciable increase in toxicity for at least an hour even when freely exposed to the air and in 5-per cent. glucose solution there is no increase in toxicity for at least four hours. On the other hand, as shown by Roth and Reid, there may be an actual decrease in toxicity after standing for ten to twenty minutes and this shows that the undue haste with which it is generally advised to prepare and inject solutions of arsphenamin in the treatment of syphilis is uncalled for and even unwarranted.

Solutions of neoarsphenamin, however, are less stable and glucose does not appear to protect this compound against color changes and an increase of toxicity as is the case with arsphenamin. For this reason this drug and sulpharsphenamin should be injected fairly promptly after solutions are made, although here again there are no reasons for undue haste as it would appear that an increase of toxicity does not occur during the first hour even when the solutions are freely exposed to air. According to the results of a few similar experiments which I have conducted with sulpharsphenamin, I believe that solutions of this compound in distilled water act in a manner similar to solutions of neoarsphenamin.

Not infrequently the question arises whether or not it is safe to keep solutions of arsphenamin, neoarsphenamin or sulpharsphenamin over a few hours or until the following day before administration. I believe that all may be safely kept for at least several hours *in narrow cylinders filled to the stoppers to prevent contact with air and* that, indeed, arsphenamin solutions may be kept in this manner for at least one or two days.

Influence of Alkalinization of Solutions of Arsphenamin, in Relation to Toxicity and Therapeutic Activity.—When commercial arsphenamin is dissolved in water or saline the solution is *acid;* when a solution of sodium hydroxid is slowly added, a heavy yellowish-white curdled precipitate is formed which dissolves upon the addition of more alkali and the addition of just enough to dissolve the precipitate to clear the solution (approximately 0.65 to 0.7 c.c. of a normal solution of NaOH per 0.1 gm. of compound) mainly forms a solution of the *monosodium* salt of arsphenamin. When about one-third more alkali is added to the clear solution totalling about 0.9 c.c. of normal NaOH solution a solution of the *disodium* salt of arsphenamin is produced.

Ehrlich found that *acid* solutions were very toxic for the lower animals when injected intravenously and likewise very painful for human beings when injected intramuscularly; he therefore warned against the use of such solutions and advocated adding to the solutions of arsphenamin, a 15-per cent. solution of sodium hydroxid, drop by drop, until the precipitate which formed was redissolved. An additional drop or two of the alkali was then added, which gave mainly a solution of the monosodium salt. Such solutions were shown to be relatively unstable in air, becoming turbid within a half hour in some cases and clinically, it was observed by others that solutions prepared by this method produced more reactions than those made more alkaline.

Experimental Observations on the Influence of Alkalinization upon the Toxicity of Arsphenamin.—The high toxicity of *acid solutions* of arsphenamin injected intravenously is

mainly due to the agglutination of erythrocytes and precipitation of blood proteins and the drug with the production of embolism and infarction described in Chapter XLV. In 1916 Schamberg, Raiziss and I showed that 0.5- to 1-per cent. solutions (equivalent to 0.6 gm. in 60 to 100 c.c.) injected intravenously in rabbits and rats were immediately toxic in doses of approximately 0.040 gm. per kilo, whereas neutralized or alkaline solutions were tolerated in doses of 0.090 to 0.100 gm. per kilo, showing that the acid solutions were 50 to 60 per cent. more toxic than the latter. Solutions of the *monosodium and disodium salts* of arsphenamin are, therefore, much less toxic than solutions of similar strength of acid solutions. Furthermore, *the disodium salt is somewhat less toxic than the monosodium salt.* For example, Danysz,[32] in 1917, concluded, on the basis of theoretical considerations and a number of experiments, that arsphenamin solutions made up with an excess of alkali were less toxic than those prepared in the usual manner. Roth[33] found that the intravenous injection of rats with 2 per cent. solutions of the monosodium salts of different lots of arsphenamin in dose of 0.1 gm. per kilo produced three to four times more deaths at the end of two and fourteen days than solutions of the disodium salts of the same compounds.

Several years ago I again studied this subject employing white rats injected intravenously at the rate of 1 c.c. per 2 minutes with 2 per cent. solutions of different brands of arsphenamin made up into three solutions as follows: (*a*) acid solutions; (*b*) solutions mainly of the monosodium salt containing just enough sodium hydroxid to dissolve the precipitates and (*c*) solutions mainly of the disodium salt containing 1 c.c. of normal sodium hydroxid for each 0.1 gm. of compound. Each solution was injected in increasing amounts and the following average figures show the largest doses per kilo of body weight proving immediately fatal and the largest amounts tolerated for 2 and 14 days respectively:

	Immediately Lethal	Maximum Tolerated Dose for 2 Days	Maximum Tolerated Dose for 14 Days
Acid solution	0.020 to 0.030	0.020	0.010 to 0.020
Monosodium solution	0.140 to 0.160	0.130 to 0.150	0.120 to 0.140
Disodium solution	0.160	0.140 to 0.150	0.110 to 0.130

These results show, in general terms, that 2-per cent. acid solutions of arsphenamin are six to eight times more toxic than neutral or slightly alkaline solutions; also that slightly alkaline solutions (disodium salt) are slightly less toxic in *two days* than neutral solutions (monosodium salt), but slightly more toxic than the latter at the end of fourteen days, confirming the observations of Lake,[34] who found that of rats injected with 0.100 gm. of arsphenamin per kilo in the form of 2-per cent. solutions neutralized with 0.75 c.c. of normal NaOH per 0.1 gm. of drug, 7.27 per cent. died in twenty-four to forty-eight hours and 5.45 per cent. after forty-eight hours, with 49.1 per cent. survivals; whereas, in a second group, injected with the same dose of the same compounds in 2-per cent. solutions alkalinized by the addition of 1.25 c.c. of normal NaOH per 0.1 gm. of drug, which was 30 per cent. in excess of the amount necessary to form the disodium salt, 3.84 per cent. died in twenty-four to forty-eight hours, 15.38 per cent. after forty-eight hours, with a survival of 44.23 per cent.

This brings up, therefore, the question of the *toxicity of hyperalkalinized solutions of arsphenamin.* Schamberg, Raiziss and I observed in experiments with rats, if one made up a solution of arsphenamin in 100 c.c. of water using just enough of a 15-per cent. solution of sodium hydroxid to neutralize the solution—that is, to the point of just dissolving the precipitate—that as much as 3 c.c. more of the alkali solution could be added to the clear solution without appreciably increasing its toxicity although the addition of 5 c.c. increased the toxicity in a definite manner. Lake has observed similar results upon the toxicity of sodium hydroxid alone for white rats by intravenous injection.

In this connection it may be mentioned that Roth has observed that the *quality of sodium hydroxid employed* may have a distinct influence on toxicity in white rats. He has lately encountered a so-called "chemically pure" sodium hydroxid, which, when made up as a normal solution, deposited, after standing several days, a considerable amount of a light brown gelatinous material. No chemical examination was made of this substance, but experiments on rats showed that solutions of arsphenamin alkalinized with the bottom portion of this sodium hydroxid solution containing the deposit was considerably more toxic (40 per cent. survivals for fourteen days) than a solution made alkaline with the top or clear portion (80 per cent. survivals for fourteen days).

Experimental Observations on the Influence of Alkalinization upon the Trypanocidal and Spirocheticidal Activity of Arsphenamin.—It is well established and quite clear, therefore, that neutral and slightly alkaline solutions

of arsphenamin are at least six to ten times less toxic than acid solutions. But how does alkalinization affect the toxicity of arsphenamin for parasites, that is, its therapeutic activity?

Ehrlich had observed that the acid solutions were distinctly more spirocheticidal (for *Sp. recurrentis*) than neutral solutions and I have observed that the same is true in regard to experimental trypanosome and *Spirocheta pallida* infections. White rats infected with *Tr. equiperdum* and injected intravenously twenty-four hours later with varying amounts of different brands of arsphenamin in 2-per cent. acid, neutral and alkaline solutions, as per the technic of my trypanocidal test, have shown the following average minimal curative doses per kilo of body weight:

Acid solutions	0.003 gm.
Neutral (mainly monosodium salt)	0.004 gm.
Alkaline (mainly disodium salt)	0.006 gm.

It will be observed, therefore, that acid solutions of arsphenamin are approximately *twice* as toxic for *Tr. equiperdum* as alkaline solutions and in a few experiments conducted with rabbits infected intratesticularly with *Sp. pallida* and treated after the development of lesions as described in Chapter XXI, essentially similar results were observed. But since alkalinization decreases toxicity for the host six to ten times, largely by reducing the acute intravascular hemoclastic reactions of agglutination, hemolysis and precipitation, no one has seriously considered the administration of acid solutions of arsphenamin even though the curative activity is at least twice that of equivalent amounts of arsphenamin in alkaline solution.

Clinical Observations on the Influence of Alkalinization of Solutions of Arsphenamin.—From the clinical standpoint, most interest centers upon the toxicity of solutions of arsphenamin alkalinized as described by Ehrlich just to the point of dissolving the precipitate and clearing the solutions as compared to those alkalinized by adding approximately one third more alkali than required for this purpose and with those rendered still more alkaline. Enough severe and even fatal reactions have resulted from the intravenous injection of acid solutions to preclude their clinical use.

Of course, it is not possible to express in figures the comparative toxicity of alkalinized solutions of arsphenamin for human beings as it is with the lower animals, but an analysis of available records in the literature shows that solutions of arsphenamin containing a slight excess of sodium hydroxid are less toxic for human beings than solutions prepared according to Ehrlich's method in which only one or two drops more of alkali are added after the solution has just cleared. For this reason Dr. McCoy, Director of the Hygienic Laboratory, has advised that each 0.1 gm. of arsphenamin be dissolved in a minimum of 20 c.c. of sterile distilled water or saline solution and neutralized with 0.9 c.c. of a normal solution of sodium hydroxid (approximately a 4-per cent. solution). However, most any strength of chemically pure sodium hydroxid from 4 to 15 per cent. may be employed if it is added to the solution in small graded amounts at a time, the physician recording in his mind the amount employed in drops or fractions of a cubic centimeter to just dissolve the precipitate and clear the solution and then adding arbitrarily one third more of this amount to the clear solution. This is probably a better method than adding the required amount of *normal* solution all at once, as 5.4 c.c. for 0.6 gm. of arsphenamin, unless one is sure that the normal solution is correct on the basis of titration. When one may rely upon the correctness of the normal solution it is permissible and perfectly good technic to arbitrarily add 0.9 to 1.0 c.c. for each 0.1 gm. of dissolved compound; otherwise, it is better to proceed as above described.

Solutions prepared in this manner do not become as quickly turbid upon standing exposed to the air and produce a lower incidence of toxic reactions. There is no advantage in adding more sodium hydroxid solution; toxicity is not reduced and the solution becomes more irritating to the veins producing

phlebitis and sometimes thrombosis. A great excess of alkali also increases the danger of intravascular reactions of hemolysis and agglutination.

Rate of Intravenous Injection of Arsphenamin and Its Substitutes in Relation to Toxicity.—As previously stated there is an intimate relation between concentration of solutions of arsphenamin and the rate of injection. In other words, a concentrated solution injected *slowly* may be as well borne as a dilute solution injected rapidly, but concentrated solutions injected rapidly certainly produce the highest incidence of toxic reactions in both man and the lower animals.

Roth has brought this out in a particularly clear and decisive manner in experiments employing guinea-pigs injected intravenously with 0.040 to 0.050 gm. of disodium arsphenamin per kilo in 2-per cent. solutions. When the dose was injected rapidly at the rate of 6 to 10 seconds, the mortality at the end of forty-eight hours was 57 per cent. and at the end of fourteen days, 67 per cent.; alternate animals injected slowly at the rate of 95 to 128 seconds, showed a mortality of only 7 per cent. at the end of forty-eight hours and 33 per cent. at the end of fourteen days. Similar results were observed with white rats injected with 0.100 gm. per kilo; among animals rapidly injected the mortality was 52 per cent. at the end of forty-eight hours and 56 per cent. at the end of two weeks in contrast with a mortality of 28 and 40 per cent. respectively among those slowly injected. From these experiments Roth concluded that the rate of administration is a potent factor in modifying the toxicity of arsphenamin, the toxicity increasing as the rate is increased. Furthermore, the depression of guinea-pigs and rats which occurs subsequent to the injection, was more pronounced after rapid than after slow injection.

Clinically, the difference between rapid and slow injections is less decisive, because the total amount of drug per kilo administered to human beings of average weight (0.6 gm. per 130 pounds) is approximately four to ten times less per body weight than employed by Roth in his experiments; but there can be no doubt of the fact that, in a broad and general manner, the rapid intravenous injection of human beings with solutions of disodium arsphenamin or silver arsphenamin, by using a large needle and gravity or by a syringe, produces more toxic reactions and especially more immediate or almost immediate reactions than slower injections requiring at least fifteen minutes for the injection of 100 c.c. or more of solution.

With solutions of neoarsphenamin, sulpharsphenamin and tryparsamid, the importance of rapid injection is much less because these compounds are very much less likely to produce acute intravascular reactions of hemolysis, agglutination and precipitation described in Chapter XLV. Even concentrated solutions of these as 0.45 to 0.9 gm. in 15 to 25 c.c. of water may be injected intravenously with a No. 22 needle at the rate of 1 or 2 minutes without increasing appreciably the incidence of toxic reactions in human beings, although I have found that both neoarsphenamin and sulpharsphenamin injected into white rats in the form of 4 per cent. solutions were distinctly better borne at the rate of 1 c.c. per two minutes than 4 c.c. per minute.

Toxic Reactions Caused by Rubber Tubing.—Of further interest in this subject of methods of administration of arsphenamin and its substitutes in relation to toxic reactions is the question raised by Stokes and Busman[35] of toxic reactions due to poisonous substances derived from rubber tubing employed in the gravity method of injection. These investigators studied the cause of an arsphenamin reaction, characterized by a chill with a sharp rise in temperature coming on from thirty minutes to one hour after intravenous injection, accompanied by nausea, vomiting, diarrhea, pain in the head and back and varying degrees of prostration. The reaction appeared in crops, so to speak, and then disappeared for a considerable period, only to recur in the midst of what appeared to be a period of flawless technical accuracy.

It was finally determined that this reaction resulted from the employment of a certain widely distributed brand of so-called pure gum rubber tubing.

The tubing, when new, appears to contain a toxic agent responsible for the reaction. The toxic substance gradually disappears from the tubing on use, and may be removed in the first instance by soaking it for six hours in normal sodium hydroxid solution and rinsing. The toxic property is not destroyed in the ordinary process of sterilization by boiling (from one-half to one hour), is not soluble in water or removable by irrigation, appears in toxic amounts in arsphenamin, neoarsphenamin and dilute sodium hydroxid solution merely on passing them through a new tube en route from container to vein, and apparently is not associated with the mechanically removable debris from the inner surface of the tube.

My friend, Dr. Walter I. Galland has discussed this interesting question at length[36] and ascribes the toxic effects largely to the presence of impurities. As stated by him "from the viewpoint of the rubber manufacturer it is possible to make rubber products free from any of the deleterious ingredients mentioned. These substances are not prerequisite to the compounding of superior rubber products, and such a product should be insisted upon. A tentative specification might be proposed in outline. Rubber goods intended for use within body cavities or in contact with body surfaces should be compounded of high grade new rubber, preferably para or plantation rubber. The use of reclaimed rubber, boiled or oxidized oils, or rubber substitute, should be prohibited. No filling ingredients containing lead, antimony, arsenic, chromium or other poisonous substance should be permitted."

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CHAPTER XLIX

ROUTE OF ADMINISTRATION OF ARSPHENAMIN, NEOARSPHENAMIN AND OTHER ORGANIC ARSENICAL COMPOUNDS IN RELATION TO TOXICITY AND THERAPEUTIC ACTIVITY

Shortly after the discovery of salvarsan and its introduction in the treatment of syphilis, the drug was administered by intramuscular injection. Solutions of the hydrochlorid in water (acid) produced so much pain and tissue necrosis that this method was abandoned in favor of intramuscular injections of alkaline solutions and Wechselmann's neutral suspension. Ehrlich observed from Hata's experiments with the lower animals that intramuscular injections gave a more continuous and intense therapeutic effect than intravenous injections although a great drawback to the routine use of the former route was the pain and disability produced. Attempts were then made to reduce the local reaction by injecting suspensions in oil and glycerin, but none of these methods served to appreciably reduce the considerable necrotic action and pain of arsphenamin and neoarsphenamin in full doses for the majority of adults.

The intravenous route, therefore, has become the usual and favorite one for the administration of arsphenamin and neoarsphenamin, not, however, because of superior therapeutic results, but primarily to render the injections free of pain and disability. Indeed, as will be shortly pointed out, the intramuscular route of administration appears to yield as good and even better therapeutic results than the intravenous route.

Arsphenamin and neoarsphenamin are also injected intraspinally and even intraventricularly for the treatment of some cases of syphilis of the central nervous system. They have also been given by mouth and by rectal injection. In some instances the powders have been applied locally to open syphilitic lesions and in this chapter it will be my purpose to discuss and compare these various routes of administration from the standpoints of toxic reactions and therapeutic results.

Intravenous Administration of Arsphenamin, Neoarsphenamin and Other Organic Arsenical Compounds in Relation to Toxicity and Therapeutic Activity.—In a broad and general manner it may be stated that intravenous injections of all of the organic arsenical compounds and especially of arsphenamin are more likely to produce toxic reactions than subcutaneous or intramuscular injections; this does not mean, however, that the latter routes are free of untoward effects aside from those of local pain and disability at the sites of injection. But in intravenous injections there are more chances of disturbing the physico-chemical equilibrium of the blood and producing toxic reactions by various physico-chemical reactions due to variations in viscosity, hydrogen-ion concentration, disturbances of acid-base equilibrium, the production of agglutination and hemolysis of erythrocytes, the formation of various precipitates, etc., already discussed with detail in Chapter XLV. Furthermore, rapid intravenous injections and especially of concentrated solutions, brings the drug in sudden contact with vital and important centers and organs and intensifies their physiological and pharmacological effects

so greatly that toxic effects may be produced. For example, the acute vasoparetic reaction or "nitritoid crisis" is usually produced by intravenous injections with sudden and immediate effects upon the vasomotor center and vasomotor nerves in hypersensitive individuals; intramuscular injections are very much less likely to produce this reaction because of slower absorption, although Milian[1] has recorded instances of "nitritoid crisis," "serous apoplexy" and acute vesicular eruptions from subcutaneous injections and regards this route of administration just as likely to produce toxic reactions as intravenous injections. Petges[2] also records the occurrence of acute reactions in 6 out of 57 patients treated with subcutaneous injections of sulfarsenol (sulpharsphenamin) although the reactions did not come on immediately as after intravenous injections, but rather several hours later which he considers an additional disadvantage, as the patient may be removed from observation and treatment.

It is true, however, that by attention to technical details in the administration of arsphenamin and neoarsphenamin the incidence of acute toxic reactions may be minimized, but the fact remains that they are more likely to occur than by intramuscular injections; *late* toxic reactions, however, as dermatitis and jaundice, are not as much influenced and, indeed, the incidence of dermatitis may be increased by accidental or purposeful subcutaneous injections.

From the standpoint of toxic reactions, the main disadvantage of intravenous injections is, therefore, the higher incidence of acute toxic reactions due to toxic physico-chemical factors of the drug, the sudden introduction of the drug, the toxicity of organic matter and other factors connected with the water or saline solution employed, etc. The main advantage is the freedom of pain and disability when intravenous injections are properly and skilfully given, and since all attempts to reduce the local necrotic effects of arsphenamin and neoarsphenamin in adult sized doses have not been generally successful, the intravenous route is to be preferred for the treatment of the majority of adults with these compounds, although small but adequate doses, and especially of neoarsphenamin, may be given to infants and young children in whom intravenous injections are difficult, impossible or dangerous (by the superior longitudinal sinus).

From the standpoint of therapeutic effects, the intravenous route is preferred in the acute early stages of syphilis for the rapid destruction of spirochetes in the lymph, blood and fixed tissues; but in the later stages, when the spirochetes are largely fixed in the tissues, the intramuscular or subcutaneous routes are to be preferred because absorption and elimination are reduced and spirocheticidal effects are actually improved probably by reason of the slower elimination which permits of the partial oxidation of larger amounts of arsphenamin and neoarsphenamin to the therapeutic oxids. At least, intramuscular or subcutaneous injections should be given from time to time alternately with intravenous injections for these more prolonged and continuous therapeutic results and it is very much desired, therefore, to have available an organic arsenical of the therapeutic activity of arsphenamin and neoarsphenamin but without the locally necrosing and painful effects of the latter by subcutaneous and intramuscular injection.

Subcutaneous and Intramuscular Injection of Arsphenamin, Neoarsphenamin, Sulpharsphenamin and Other Organic Arsenical Compounds in Relation to Toxicity and Therapeutic Activity. *Local Reactions Produced by Arsphenamin and Neoarsphenamin.*—From the standpoint of toxic reactions, the main advantage of subcutaneous and intravenous injections is the greater freedom from acute reactions, although it cannot be stated by any means that

acute and dangerous reactions are eliminated by these routes of administration. The main disadvantages are the local necrosing effects with resulting pain and disability, which may be quite considerable with the trivalent arsenicals as arsphenamin and neoarsphenamin, and various methods have been advocated for the subcutaneous and intramuscular injections of these compounds. Solutions of arsphenamin hydrochlorid in water (acid solutions) were among the first injected, but were quickly abandoned because of the very severe local reactions, in favor of Wechselmann's neutral suspension and alkaline solutions. Suspensions of arsphenamin were then advocated by Levy-Bing and Lafay,[3] Kromayer,[4] Pollitzer,[5] Hazen[6] and others, but abscesses and other severe reactions occurred, as reported by Hazen, by these injections as well as from suspensions of neoarsphenamin in glycerin advocated by Wolbarst.[7] More recently, however, some physicians of wide experience as Wechselmann,[8] Harrison, White and Mills,[9] Fordyce and Rosen[10] and others have developed methods for the subcutaneous and intramuscular injections of neoarsphenamin, which are said to produce local reactions not worse than the severe reactions produced by intramuscular injections of calomel and Sutton[11] has recently developed a method for intramuscular injections of arsphenamin. These methods will be described in Chapter LX, but here I may state that my experience with all of them has shown that most adults object to a series of injections if the doses are 0.3 gm. or over although a series of injections of smaller amounts of neoarsphenamin can usually be given to young children and infants with considerable success.

Local and General Reactions Produced by Sulpharsphenamin.—Within recent years, a new compound designated as sulfarsenol or sulpharsphenamin has been found to produce much less local reaction by subcutaneous or intramuscular injection. Preliminary reports by Levy-Bing and his associates,[12] Yernaux and Bernard,[13] Doble,[14] Crawford and Fleming[15] and others have indicated that this compound injected intramuscularly produces much less local irritation than either arsphenamin or neoarsphenamin; Emery and Morin[16] believe that subcutaneous injections are even better borne than intramuscular injections. However, according to Crawford and Fleming, the drug has not produced as good therapeutic results as arsphenamin in congenital syphilis; Durveux,[17] on the other hand, states that the serological results are jus as good and I will discuss this phase later with more detail.

My own experience with two brands of American made sulpharsphenamin have shown that this compound is generally better borne and especially by children, than either arsphenamin or neoarsphenamin and probably, better by subcutaneous than by intramuscular injection, although in some patients the reactions produced by subcutaneous and intramuscular injections of doses of 0.6 gm. dissolved in 2 to 5 c.c. of sterile water have been so severe that a series of injections could not be given. In other words, adult human beings in my experience vary greatly in their local reactions to this drug; in some individuals even small doses as 0.3 gm. have produced severe reactions while in others doses of 0.6 gm. were well borne although in no instance free of some reaction. The differences are not due altogether to the technic of injection, but to biological factors as well, and I have the impression that the local reactions are sometimes worse if large doses of the iodids are being given at the same time, as will be shortly discussed with more detail.

As previously stated, subcutaneous and intramuscular injections are not free of general toxic reactions similar to those produced by intravenous injections but developing later and more slowly. Dermatitis and jaundice may be produced as well as the more acute symptoms of nausea, vomiting,

diarrhea, etc., although the incidence of general reactions is lower than when these compounds are given intravenously.

Local Reactions Produced by Pentavalent Arsenical Compounds.—Pentavalent arsenicals as atoxyl, tryparsamid, amino-arsenophenol or "eparseno" (3:3′ diamino—4:4′ dioxy—arsenobenzene or Pomaret's "No. 132"), as a group produce less local reaction upon subcutaneous or intramuscular injection than the trivalent arsenicals. They are likewise less therapeutically active by both intravenous and intramuscular injection and must be given therefore in larger doses which usually results in local pain and some disability. Jeanseline, Pomaret and Bloch[18] have given as much as 0.12 gm. in 1 c.c. of Pomaret's amino-arsenophenol every other day for 25 consecutive injections by intramuscular injections high in the gluteal region.

Comparative Toxicity of the Organic Arsenical Compounds for the Lower Animals Administered by Intravenous, Intramuscular and Subcutaneous Injection.—While in human subjects arsphenamin, neoarsphenamin, sulpharsphenamin and other medicinal organic compounds of arsenic are less likely to produce general toxic reactions when given subcutaneously or intramuscularly than by intravenous injection, yet in the lower animals neoarsphenamin is more toxic by subcutaneous or intramuscular injection than by intravenous injection. This curious fact was first shown by Castelli[18] and confirmed by Schamberg, Raiziss and myself.[19] We observed that, while the highest average tolerated dose of neoarsphenamin for the rat by subcutaneous injection was 0.177 gm. per kilo, by intravenous injection it was 0.254 gm. per kilo. Arsphenamin, however, was always more toxic by intravenous than by intramuscular injection. It is difficult to explain this peculiarity of neoarsphenamin, unless it is due to the fact that when injected intravenously the drug is rapidly eliminated and therefore borne in higher doses than by subcutaneous or intramuscular injection when elimination is much slower although reduction by partial oxidation to the toxic oxids of the compounds in the tissues is much faster than occur with arsphenamin.

Sulfarsenol, or sulpharsphenamin, is likewise slightly less toxic by subcutaneous than by intravenous injection according to Voegtlin, Dyer and Johnson.[20] I have also found sulpharsphenamin slightly less toxic by subcutaneous injection, although the differences were not very great and frequently the maximal tolerated doses for rats were about identical.

Comparative Therapeutic Activity of Arsphenamin, Neoarsphenamin and Sulpharsphenamin by Intravenous, Intramuscular and Subcutaneous Injection.—Of almost equal interest and importance is the comparative therapeutic effects of the various medicinal compounds of arsenic by different routes of administration. When injected intravenously, elimination is ordinarily so rapid that one would expect a greater degree of therapeutic activity by subcutaneous or intramuscular injections of solutions of the compounds in a rapidly absorbable state and experiments with the lower animals have shown quite conclusively that with some compounds this is actually the case.

Castelli, for example, referred to above, found, in experiments with mice infected with *Sp. recurrentis*, that the smallest sterilizing dose of arsphenamin by both subcutaneous and intravenous injection was 0.0106 gm. per kilo, but the dose of neoarsphenamin by subcutaneous injection was 0.0166 gm. and by intravenous injection, 0.0199 gm. per kilo.

Voegthin and his associates have found sulpharsphenamin slightly more trypanocidal by subcutaneous than by intravenous injection and with arsphenamin and neoarsphenamin, Voegthin and Smith[21] state that the trypanocidal effects were as good after intramuscular as after intravenous injections.

My trypanocidal tests, conducted with rats infected with *Tr. equiperdum* and tested by Voegthin's method, have yielded similar results, not only with sulpharsphenamin but with neoarsphenamin and the disodium salt of arsphenamin as well, these results being summarized as follows and showing the smallest effective doses per kilogram of weight.

	Arsphenamin	Neoarsphenamin	Sulpharsphenamin
By intravenous injection.....	0.006 gm.	0.012 gm.	0.026 gm.
By intramuscular injection.....	0.004 gm.	0.008 gm.	0.018 gm.
By subcutaneous injection.....	0.004 gm.	0.008 gm.	0.022 gm.

It will be noted that intramuscular and subcutaneous injections of all three of these compounds yielded better trypanocidal effects than equivalent amounts injected intravenously; sometimes, and especially with sulpharsphenamin, slightly better results were observed after intramuscular than after subcutaneous injections. But it is also worthy of note that sulpharsphenamin is distinctly less trypanocidal than arsphenamin and neoarsphenamin; apparently those chemical alterations resulting in a decrease of its local toxic or organotropic effects also reduced its therapeutic activity.

Similar experiments employing rabbits injected with *Sp. pallida* have yielded somewhat similar results. In one of these tests the approximately smallest single doses of the three compounds per kilo of weight administered intravenously and intramuscularly, required to bring about a disappearance of the local scrotal lesions and sterilization of the lymphatic glands, were as follows:

	Arsphenamin	Neoarsphenamin	Sulpharsphenamin
By intravenous injection.....	0.014 gm.	0.018 gm.	0.030 gm.
By intramuscular injection.....	0.011 gm.	0.014 gm.	0.026 gm.

The discovery of a new arsenical compound more spirocheticidal than sulpharsphenamin but less irritating for the subcutaneous tissues and muscles, or at least, not more irritating, is very much needed for the treatment of syphilis and especially for chronic syphilis. Sulpharsphenamin is a great improvement over arsphenamin and neoarsphenamin for subcutaneous or intramuscular injection from the standpoint of local reactions, but is so inferior in curative activity that I believe it should not supersede either drug by intravenous injection except in those cases when intravenous injections cannot be given to obese adults or young children. Probably, the restrictions should not be as stringent as stated; in the treatment of chronic syphilis of adults to whom intravenous injections are very easy, it is frequently my plan to give a subcutaneous or intramuscular injection of sulpharsphenamin every third or fourth injection in order to prolong the effects and to permit the local tenderness to entirely subside before subsequent injections are given.

From the serological standpoint, intramuscular injections of arsphenamin have sometimes yielded better results than intravenous injections, alkaline solutions being employed in both, as reported by Craig;[22] Colonel Harrison states that in the British Army the serological results were better following intramuscular injections of neoarsphenamin than after intravenous injections.

Leonard[23] reports that in 511 patients treated with seven injections of neoarsphenamin and seven of mercury, the percentage of negative Wassermann reactions was higher after intramuscular than after intravenous injections and states that the former route of injection yielded excellent clinical results.

Stokes and others have reported satisfactory clinical results from subcutaneous and intramuscular injections of sulpharsphenamin. As for myself, it must be stated that in primary and secondary syphilis I have observed a more rapid disappearance of lesions and spirochetes from intravenous injections of 0.9 gm. neoarsphenamin than from intramuscular and subcutaneous injections of 0.4 to 0.6 gm. sulpharsphenamin. As stated by Tzanck and Dohen,[24] sulpharsphenamin by subcutaneous injection is valuable in the treatment of congenital syphilis and in patients with advanced and grave organic lesions, such as arteriosclerosis, aortitis or nephritis, but in the primary stage of syphilis and especially in the period of golden therapeutic opportunity before the Wassermann reaction has become positive, the intravenous route is always to be preferred; for myself, when intravenous therapy is indicated and especially in the treatment of acute early syphilis, I much prefer a good brand of neoarsphenamin, because the toxic effects are no greater and indeed usually less, while the therapeutic effects are much better.

Local Tissue Reactions Produced by Intramuscular Injections of Arsphenamin, Neoarsphenamin, and Sulpharsphenamin.—In conclusion, it may be well to briefly consider in a comparative manner the kind and degree of local tissue reactions produced by intramuscular injections of arsphenamin and its substitutes. It is well known, of course, that intramuscular injections of arsphenamin in neutral suspension, as originally employed by Wechselmann, in alkaline solution and suspended in oil, are more painful and produce larger and severer local reactions in human beings than intramuscular injections of similar doses of neoarsphenamin dissolved in an equal volume of water or suspended in the same amount of oil.

Swift[25] and others have shown in histological studies of the lesions excised from the lower animals that the difference in microscope appearance of the necrotic areas resulting from intramuscular arsphenamin and neoarsphenamin injections was quite striking. Following the arsphenamin injection, the necrosis and polymorphonuclear infiltration were most prominent, while following the neoarsphenamin injections the inflammation was of a chronic type, and the area was less sharply differentiated from the surrounding tissue.

I have endeavored to make a comparative study of the histological changes in guinea-pigs and rats produced by arsphenamin, neoarsphenamin and sulpharsphenamin, dissolved in water and suspended in oil, and injected intramuscularly. The amounts of drug injected have been mostly 0.025 gm. in 0.5 c.c. of menstrum for guinea-pigs; in some experiments employing rats the dose was 0.002 gm. in 0.2 c.c. of menstrum. The technical difficulties proved quite considerable from the standpoint of placing the study on a comparative basis, mainly because of the difficulties encountered in depositing the drugs in the same muscles in order to equalize as far as possible the effects of massage from the movements of the animals, as well as the subsequent removal of the lesions with sections through approximately the same places. Only by studying a large number of serial sections could one arrive at an approximate estimate of the comparative degrees of irritation produced. Sterile cotton seed oil was employed because I believe a vegetable oil is to be preferred to a mineral oil like paraffin oil, since saponification occurs in the tissues with better absorption and less chances of encapulation.

In the first place, intramuscular injections of sterile oil alone produces some histological changes characterized by slight degrees of muscle necrosis

and polymorphonuclear leucocytic infiltration; indeed, intramuscular injections of sterile distilled water alone have produced slight degrees of irritation and injections of alkaline water corresponding to the alkalinity of solutions of the disodium salt of arsphenamin, have produced considerable degrees of irritation characterized by muscle necrosis and leucocytic infiltration.

In a broad and general manner, the histological changes produced by all three of these arsenicals were quite similar, although varying in degree. All produce necrosis of muscle in contact with the deposited drug and all exert a positive chemotaxis upon polymorphonuclear leukocytes, although the leukocytes may not penetrate to the deposited drug and especially in the case of

FIG. 21.—LOCAL REACTION PRODUCED BY INTRAMUSCULAR INJECTION OF ARSPHENAMIN IN OIL.

Injection of 0.025 gm. of arsphenamin in 0.5 c.c. of sterile cotton-seed oil (5 days).

arsphenamin. Coagulation and liquefaction necrosis may occur and in lesions more than a week old before excision, evidences of fibrosis and encapsulation were evident. Sulpharsphenamin appeared to produce vascular necrosis with interstitial hemorrhages, more frequently than either arsphenamin or neoarsphenamin.

Figures 21 to 24 show in a general manner the comparative degrees of tissue injury produced by similar doses of these compounds. Solutions of the disodium salt of arsphenamin in water always produced more injury than solutions of neoarsphenamin and sulpharsphenamin in water. As

a general rule solutions of neoarsphenamin produced severer injury than solutions of sulpharsphenamin but this was not always the case. Indeed in many animals the sulpharsphenamin injury was slightly more severe than the neoarsphenamin injury, although human beings generally experience less discomfort and pain from intramuscular injections of the latter than from the former. Histologically, however, the degree of injury produced in guinea pigs and rats was frequently similar to that produced by neoarsphenamin and in several instances, the degree of vascular injury was more marked.

As a general rule suspensions of all three compounds in oil produced greater degrees of injury than solutions in water. I realize that this state-

FIG. 22.—LOCAL REACTION PRODUCED BY INTRAMUSCULAR INJECTION OF ARSPHENAMIN IN WATER.

Injection of 0.025 gm. of disodium arsphenamin in 0.5 c.c. sterile water (5 days).

ment is not in harmony with clinical experience, but nevertheless, the changes as observed showed that in suspensions in oil, the drugs themselves could usually be found in the tissues surrounded by greater degrees of muscle liquefaction and wider zones of leucocytic infiltration. It may be that the oil protects more against pain, but the size and severity of the local reaction was seldom less than observed with water solutions and sometimes greater. In other words, solutions of all three compounds in water produce more diffuse lesions probably because the drug is more rapidly diffused in the muscles; suspensions in oil produce more localized lesions, but since the

concentration of drug acting upon the muscle at the site of injection is greater, because diffusion is less, the degree of necrosis is usually greater with more evidences of liquefaction necrosis.

Influence of Iodids upon Intramuscular Reactions.—In a second series of experiments animals were given potassium iodid by mouth in dose of 0.02 gm. per kilo, corresponding to 1.2 gm. or 20 grains per 132 pounds, for six days before the drugs were injected intramuscularly. The resulting lesions frequently showed a greater degree of polymorphonuclear leucocyte infiltration and liquefaction necrosis than the animals not receiving iodid.

FIG. 23.—LOCAL REACTION PRODUCED BY INTRAMUSCULAR INJECTION OF NEOARSPHENAMIN IN OIL.

Injection of 0.025 gm. of neoarsphenamin in 0.5 c.c. sterile cotton-seed oil (5 days).

In conducting these experiments, smaller doses of the three compounds were injected in order to bring out more sharply the possible influence of the iodid administered. It is well known that intracutaneous injections of "luetin," agar, etc., produce larger and more pustular reactions in human beings under potassium iodid treatment than among those who have not taken this drug, as discussed with detail in Chapter LVII. These experiments indicate that intramuscular injections of the arsenicals in human beings taking potassium iodid may produce larger local reactions because iodin appears to favor suppuration, probably by increasing the activity of proteolytic enzymes derived from devitalized muscle and connective tissue cells

and leukocytes by inhibiting the influence of the antiferments, which, according to Jobling, Eggstein and Peterson, are apparently the unsaturated fatty acids of the body fluids. Similar experiments with intramuscular injections of various salts of mercury and potassium tartrobismuthate have yielded similar results as described in Chapters LIII and LV. I am not sure that, clinically, one may observe a greater degree of local reaction from intramuscular injections of various antisyphilitic drugs among individuals taking potassium or sodium iodid, because the injections are quite deep and differences in reaction may not become apparent, but these histological studies employing

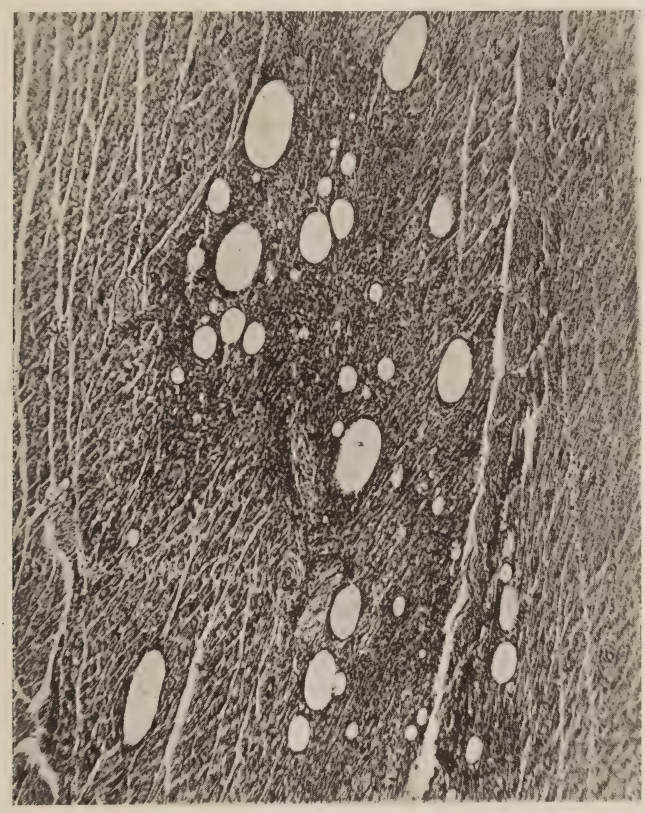

FIG. 24.—LOCAL REACTION PRODUCED BY INTRAMUSCULAR INJECTION OF SULPHARSPHENAMIN IN WATER.

Injection of 0.025 gm. of sulpharsphenamin in 0.5 c.c. of sterile water (5 days).

guinea-pigs and rats have shown that iodin tends to intensify the degree of local reaction.

Intraspinal, Intraperitoneal and Intrapleural Administration of Arsphenamin and Neoarsphenamin in Relation to Toxic Reactions and Therapeutic Activity.—When solutions of arsphenamin and neoarsphenamin are applied to open mucous membranes as those of the nose, mouth and pharynx, irritation is not ordinarily produced, but when injected intraspinally, even minute amounts are capable of eliciting rather sharp reactions in the pia and subarachanoid membranes. Intrapleural injections are likewise less well borne than intravenous injections, whereas intraperitoneal injections are

well borne as shown in the following results of my experiments employing rats and rabbits with particular reference to the average maximum tolerated doses of arsphenamin (disodium salt) and neoarsphenamin per kilo of body weight by these different routes of administration:

Route	Arsphenamin	Neoarsphenamin
Subcutaneous	0.250 gm.	0.150 gm.
Intravenous	0.120 gm.	0.250 gm.
Intraperitoneal	0.200 gm.	0.280 gm.
Intrapleural	0.080 gm.	0.080 gm.
Intraspinal	0.015 gm.	0.030 gm.

In comparison with the maximum tolerated doses by intravenous administration, it will be observed that by intraperitoneal injections the doses are appreciably greater, whereas by intrapleural injection they are about one third less and by intraspinal injection about one tenth less.

From the standpoint of therapeutic activity, the results of trypanocidal tests have been equally interesting and instructive. For example, in rats infected intraperitoneally with *Tr. equiperdum* by my method and treated twenty-four hours later, the smallest curative doses per kilo of body weight by these different routes of administration were as follows:

Route	Arsphenamin	Neoarsphenamin
Intravenous	0.005 gm.	0.012 gm.
Intraperitoneal	0.004 gm.	0.014 gm.
Intrapleural	0.007 gm.	0.016 gm.

The degree of tissue reaction as shown by the amount of congestion and serous and cellular reaction, is also greater in the subarachnoid and pleural spaces than in the peritoneal cavity, but doubtless irritation of important nerves and centers adds greatly to the low tolerance for both drugs injected intraspinally. Even though well diluted with spinal fluid, the intraspinal injection of human beings with amounts of arsphenamin in excess of 0.0005 gm. (½ mg.) or 0.002 gm. (2 mg.) of neoarsphenamin are likely to produce well-marked irritation and especially of the lower cord in the neighborhood of the site of injection, as shown by shooting pains in the legs and sometimes disturbances of both bladder and rectum. I have never seen in human subjects disturbances of the respiratory and cardiac centers, but such have been recorded in the literature and great care must be exercised in the intraspinal injection of these compounds. When administered the doses should seldom exceed 0.001 gm. (1 mg.) of arsphenamin and 0.002 gm. (2 mgs) of neoarsphenamin and one half these doses are the usual amounts; they should be well diluted with serum or saline solution and, finally and best of all, well diluted with 20 to 30 c.c. of the patient's own spinal fluid before slow administration by gravity injection.

I have discussed in Chapter LXXVI the therapeutic activity and indications for intraspinal therapy in neurosyphilis. The question is frequently raised whether the very minute amounts of arsphenamin carried over in 20 to 40 c.c. of serum, secured from blood drawn one half hour after the intra-

venous injection of 0.6 gm. of arsphenamin in the Swift-Ellis method, were sufficient for exerting spirocheticidal effects and whether the addition of 0.0005 gm. (½ mg.) of arsphenamin to 10 c.c. of serum in the Ogilvie method is sufficient for exerting spirocheticidal effects and especially when it is considered that both injections are still further diluted by at least 20 c.c. of spinal fluid. Studies *in vitro* summarized in Chapter XXVIII, show that arsphenamin and neoarsphenamin must be present in concentration of at least 1:1000 to destroy *Sp. pallida* and in the Ogilvie method the final dilution of 0.0005 gm. in 10 c.c. of serum and 20 c.c. of spinal fluid is at least 1:50,000, while in the Swift-Ellis method the dilution is still higher. Unquestionably, however, spirocheticidal activity *in vivo* is higher than *in vitro*, but it is almost certain that the minute amounts of arsphenamin and neoarsphenamin introduced into the spinal fluid by these methods are insufficient for exerting direct spirocheticidal effects; the therapeutic results are due to other changes. I know from the experiments summarized above with rabbits infected intraspinally with *Tr. equiperdum* that doses of these sizes are without appreciable trypanocidal effects. It is possible that some spirochetes on the surface of the meninges may be destroyed, but I very much doubt that organisms in the substance of the cord and brain are appreciably affected in intraspinal therapy by the drugs in the spinal fluid, although the resulting hyperemia and other changes induced in both meninges and substance of brain and cord may exert therapeutic effects, as discussed in Chapter LXXVI with more detail.

Intrapleural injections are without clinical significance or application, but intraperitoneal injections of neoarsphenamin may be of service in the treatment of infantile syphilis as indicated by recent case reports by Rosenberg and others and especially since there is an increasing tendency among pediatrists to give intraperitoneal injections of saline, blood, glucose, etc.

Oral and Rectal Administration of Arsphenamin and Neoarsphenamin in Relation to Toxicity and Therapeutic Activity.—As shown by Schamberg and myself the administration of arsphenamin by mouth exerts trypanocidal effects in rats and especially when the drug is given in solution or in capsules. The doses required for decisive effects, however, must be quite large as compared with intravenous injections. For example, the smallest trypanocidal dose of arsphenamin by intravenous injection of rats infected and treated according to the technic of my trypanocidal test, was about 0.005 gm. per kilo, but when the drug was given in solution by stomach tube at least 0.060 to 0.100 gm. per kilo were required, these amounts being, therefore, at least 15 to 25 times greater than required by intravenous injection. Rabbits given daily doses of arsphenamin equivalent approximately to 0.005 gm. per kilo or 0.3 gm. per 132 pounds for 29 to 31 days in succession showed no evidence of toxicity or histological changes in the internal organs; similar results were observed in experiments with dogs given pills and capsules of arsphenamin. Human subjects have been given 0.030 gm. (½ grain) of arsphenamin in capsules three times a day for many weeks without producing disturbing symptoms except mild digestive distress, and this is only a relatively small proportion of cases.

The oral administration of arsphenamin, therefore, exerts some curative activity as we have observed in the treatment of some cases of syphilis by this route of administration; untoward or toxic effects are seldom observed. Dr. Schamberg and I have never advocated the routine administration of the drug by this route, but we believe that it sometimes serves a useful purpose for the treatment of those patients who for various reasons cannot take the drug by intravenous infusion or intramuscular injection.

Within recent years many reports have appeared upon the rectal administration of arsphenamin and neoarsphenamin. Undoubtedly absorption occurs with therapeutic effects but only when very large amounts are given. With rats infected with *Tr. equiperdum* and treated twenty-four hours later by rectal injections, I found that the smallest curative dose was approximately 0.090 to 0.110 gm. per kilo for arsphenamin and 0.150 to 0.200 gm. per kilo for neoarsphenamin, these doses being therefore about 20 times greater than the smallest curative doses by intravenous injection.

Neoarsphenamin produces less irritation of the bowels than arsphenamin and when given in solution is the drug of choice; small doses in supppositories as 0.1 gm. of either are well borne when given every other day. Suspensions in a vegetable oil produce somewhat less irritation than solutions in water. Toxic effects as nausea and vomiting are of rare occurrence.

In the treatment of syphilis of individuals to whom intravenous injections cannot be readily given and who refuse intramuscular injections, as obese and nervous women or individuals who develop acute reactions, but to whom it is desired to administer arsphenamin or neoarsphenamin, rectal administrations are worthy of trial and it is easy to teach such individuals how to dissolve and administer neoarsphenamin to themselves since aseptic precautions are not required. Such indications, however, are exceptional and the cost of the large doses required is also prohibitive in many instances.

Local Application of Arsphenamin and Neoarsphenamin in Relation to Toxicity and Therapeutic Activity.—Unquestionably both arsphenamin and neoarsphenamin exert spirocheticidal effects when brought in contact with *Sp. pallida* and *Sp. pertenuis* in chancres, mucous patches and other syphilitic and frambesial ulcers, as well as upon other spirochetes, as in spirochetic gingivitis and Vincent's angina. Under the microscope final dilutions of arsphenamin with these secretions as high as 1:1000, destroy motility and kill the parasites as shown in cultural experiments. I have observed that 1-per cent. ointments of arsphenamin applied within one hour to inoculations of *Sp. pallida* to the scrotal skin of rabbits may prevent infection and the application of 1- to 4-per cent. solutions to the gums and pharynx undoubtedly exert curative effects in spirochetic gingivitis and Vincent's angina.

Solutions of both drugs and even the powders themselves applied to ulcers, the gums and pharynx are usually free of irritative effects probably on account of the secretions and character of the tissues. This is in sharp contrast with the irritation produced when solutions are injected into closed serous spaces as the subarachanoid, pleural and peritoneal cavities.

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CHAPTER L

PROPHYLAXIS OF TOXIC REACTIONS PRODUCED BY ARSPHENAMIN, NEOARSPHENAMIN AND OTHER ORGANIC ARSENICAL COMPOUNDS AND THE CONTRAINDICATIONS TO THEIR ADMINISTRATION

From the standpoint of the prophylaxis of toxic reactions following the administration of arsphenamin, neoarsphenamin and other remove organic compounds of arsenic, most interest is in relation to their intravenous injection, because this route is more likely to produce untoward effects than the subcutaneous and intramuscular routes. With the latter the chief object is to minimize the degree of local reaction of pain and disability, although general reactions and even the acute vasoparetic reaction, or "nitritoid crisis," may follow intramuscular and subcutaneous injections.

When arsphenamin or neoarsphenamin are injected intraspinally or intra-ventricularly, the chief concern is to minimize the degree of irritation of the meninges and the neighboring tissues; also to guard against excessive elevation of cerebrospinal fluid pressure. The various technical procedures to be employed for both of these purposes, as well as the reduction of pain and disability following subcutaneous and intramuscular injections, will be considered in Chapter LXII, devoted to the technic of administration of these compounds.

The question of the prophylaxis of toxic reactions following the oral and rectal administration of arsphenamin and neoarsphenamin hardly requires consideration, because these methods are not, or at least, should not be, employed except in very exceptional cases and nothing of special interest can be written of the prophylaxis of the reactions which they may produce as these effects are largely referable to direct irritation of the mucous membranes which subside as treatment is discontinued.

Our main interest in this chapter, therefore, refers to the prophylaxis of the general toxic reactions when the organic arsenicals are administered by intravenous, intramuscular and subcutaneous injection and especially in relation to intravenous injections, because with this route, we must deal with reactions due to the physical chemistry of solutions of the compounds and the acute changes that may occur in the blood stream as a result of the sudden introduction of solutions of the drugs, as well as the effects upon the fixed tissues. With subcutaneous and intramuscular injections, absorption is much slower and the incidence of toxic reactions (aside from local pain and disability) much less.

General Measures for the Prophylaxis of Toxic Reactions.—Since toxic reactions may be due primarily to the drug, to the patient or to the method

of administration as summarized in Chapter XLIV, so may the various prophylactic measures be grouped under the same three divisions as follows:

1. Referable to the Drug
   - (1) Avoid stale products (over 2 years since manufacture).
   - (2) Do not use drugs in cracked ampules.
   - (3) Do not use drugs of changed color or with strong odor.
   - (4) Do not use difficultly soluble neoarsphenamin.
   - (5) Do not inject any solution unless perfectly clear.
   - (6) Examine labels carefully to prevent the administration of arsphenamin in mistake for neoarsphenamin or sulpharsphenamin.

2. Referable to the Preparation and Administration of Solutions
   - (1) Use only freshly distilled sterile water or saline free of organic matter, or distilled water hermetically sealed against contamination.
   - (2) For dilute solutions (50 c.c. or more) use saline in preference to water.
   - (3) Avoid hot water for neoarsphenamin and sulpharsphenamin.
   - (4) Dissolve drug with minimum of shaking.
   - (5) For arsphenamin use preferably 20 to 30 c.c. of solution for each 0.1 gm. of drug.
   - (6) For arsphenamin use ⅓ excess of sodium hydroxid or 0.9 c.c. of a *normal* solution for each 0.1 gm.
   - (7) Filter all solutions.
   - (8) Allow solutions of arsphenamin to stand 10 to 20 minutes before injection.
   - (9) Inject slowly using at least 2 minutes for each 0.1 gm. of arsphenamin injected in dilute solution by gravity. For concentrated solutions of neoarsphenamin and sulpharsphenamin (15 c.c.) use at least one half minute for each 0.2 gm. of drug.
   - (10) For gravity administration avoid new rubber tubing unless previously prepared.

3. Referable to the Patient
   - (1) Allay fear and nervousness.
   - (2) Avoid undue haste; avoid operating rooms and a display of instruments, etc.
   - (3) Releave constipation.
   - (4) Inject one to several hours after last meal.
   - (5) Avoid injection during menstrual period.
   - (6) Give preliminary attention to the condition of the teeth, tonsils and other possible foci of infection.
   - (7) Examine urine for the condition of the kidneys and especially if mercury or bismuth have been given.
   - (8) Estimate functional capacity of liver in selected cases.
   - (9) Give but ½ to ¼ usual amount for first dose.
   - (10) In chronic syphilis start treatment with mercury, bismuth or with small doses of the arsenicals to prevent Herxheimer reactions.
   - (11) Exercise special care in cases of pregnancy as severe reactions may produce abortion or miscarriage.
   - (12) When administering pentavalent compounds as atoxyl or tryparsamid, have the vision and eye grounds examined repeatedly to guard against toxic amblyopias.
   - (13) Give intramuscular injections deep in the muscles; avoid injections in fatty tissues and in the neighborhood of large nerves.
   - (14) Give patients likely to develop acute vasoparetic reactions $\frac{1}{50}$th grain of atropin alone or in conjunction with a small injection of the drug ($\frac{1}{10}$th the dose) about one hour before the main injection.

All except the last mentioned of these factors have been discussed in preceding chapters and need not here receive further attention. *Since arsphenamin tends to produce more reactions than either neoarsphenamin or sulpharsphenamin, the subject of prophylaxis is of special interest in relation to the administration of this compound* and, as previously stated, the subject is

of more importance in relation to intravenous than intramuscular and subcutaneous injections.

Special Measures for the Prophylaxis of Acute Toxic Reactions.—Various additional measures have been proposed for the prophylaxis of acute reactions following intravenous injections of arsphenamin or neoarsphenamin and especially the former for the purpose of permitting the treatment of those exceptional cases of syphilis peculiarly hypersensitive to these drugs and likely to develop acute reactions on the table or immediately after injection.

I have already discussed in Chapter XLVI the injection of arsphenamin and neoarsphenamin in sterile glucose solution for this purpose. Glucose evidently delays or prevents the reduction and oxidation of the compounds and lowers especially the toxicity of arsphenamin. I have commonly employed a sterile isotonic 5-per cent. solution of chemicals pure glucose in distilled water for the administration of arsphenamin and concentrated solution (0.4 gm. in 25 c.c.) and believe that it aids in preventing untoward effects. Pranter,[1] Steinberg,[2] Dulrot,[3] Cheinisse[4] and others abroad, however, have recommended the use of hypertonic solutions (10- to 50-per cent.) with apparent success. As a general rule, about 30 c.c. of 20- to 30-per cent. solutions of Merck's glucose in distilled water have been employed and Steinberg states that the spirocheticidal effects are increased in addition to a reduction in toxicity. I doubt the correctness of this observation and rather suspect that carefully controlled experiments would show a reduction in therapeutic activity by solutions of these concentrations. Cheinisse states that glucose solutions also reduce the incidence of arsenical dematitis and jaundice and their use is worthy of serious consideration for the administration of arsphenamin and its substitutes to difficult patients, that is, individuals who have shown signs of a high degree of intolerance for these compounds.

An excellent drug for preventing the acute vasoparetic reaction is adrenalin in dose of 2 to 8 minims of 1:1000 solution by subcutaneous injection just before or during the injection of arsphenamin as advocated by Berman[5] and others. But great care must be exercised, as otherwise a vascular shock may be produced almost as dangerous as the arsenical reaction itself, as observed Stokes. Adrenalin however, is our most valuable remedy for the treatment of the acute vasoparetic reaction ("nitritoid crisis") and I believe should be reserved for this purpose rather than administered prophylactically.

Stokes[6] and Busman[7] have advised the subcutaneous injection of $\frac{1}{50}$th grain of atropin sulphate about 20 minutes before the injection of arsphenamin. Smaller doses have proven ineffective for adult cases. In their experience atropin has controlled the immediate reactions and permitted a continuation of arsenical treatment of a number of cases. I have used the drug in the type of cases described by Stokes and Busman with success and believe that it is a good plan to inject $\frac{1}{50}$th grain before the injection of arsphenamin or neoarsphenamin whenever an acute reaction is feared and to have 0.5 c.c. of 1:1000 adrenalin ready for injection if the atropin fails to check the arsenical reaction.

Tzanck and Radot[10] advise giving 0.1 gm. of neoarsphenamin by mouth one-half, one or even twenty-four hours before intravenous injection as a means of prophylaxis; I have had no experience with this method. Kenedy[11] advises administering neoarsphenamin dissolved in 10 c.c. of a 10-per cent. solution of calcium chlorid in water but my experience with this method has been bad owing to the toxic reactions sometimes produced by calcium. Sicard has advised the preliminary intravenous injection of 30 c.c. of saline containing 0.6 to 0.75 gm. of sodium carbonate on the basis of the Danysz

theory that the acute reactions are due to changes in the inorganic constituents of the blood. This method, however, has not proven successful in the experience of others.

The Prophylactic Effects of a Small Preliminary Injection of the Arsenical Compound.—Of special interest in connection with this subject of the prophylaxis of the acute immediate arsenical reactions are the effects of a small preliminary intravenous injection of the drug for the purpose of "desensitizing" the patient in a manner analogous to the subcutaneous injection of 1 c.c. of horse serum one hour before the intravenous injection of antipneumococcus or other immune serum. Danysz[12] has mentioned this subject several years ago in connection with his experimental work and more recently Kolle[13] has stated that preliminary "vaccination" injections reduce the toxicity of arsphenamin for the lower animals. Stokes and Busman, previously referred to, have likewise found the method of use clinically either alone or in combination with the subcutaneous injection of $\frac{1}{50}$th grain of atropin and Busman has employed preliminary intravenous injections of $\frac{1}{10}$th the prescribed dose one hour before the main injections, with apparent success in a series of cases.

It is very difficult to evaluate these various prophylactic methods by experiments with the lower animals, because they apparently do not develop acute reactions similar to the "nitritoid crisis" of human beings, although it is well known that rats vary in susceptibility to the arsenical compounds. But in several experiments I have observed that the preliminary or "vaccinating" injections of arsphenamin and neoarsphenamin have apparently increased the tolerance of rats for these compounds administered by the usual method described in Chapter II, as shown in the following summary. In other experiments no influence upon toxicity was apparent and the results were usually more definite with arsphenamin than with neoarsphenamin:

(*a*) The maximum tolerated dose of a solution of the disodium salt of a lot of arsphenamin was between 0.100 and 0.110 gm. per kilo; when administered one to five minutes after the preliminary intravenous injection of 0.001 gm. per kilo the maximum tolerated dose was raised to 0.140 to 0.150 gm. per kilo.

(*b*) The preliminary injection of 0.005 gm. of a lot of disodium arsphenamin one minute before raised the maximum tolerance from about 0.100 gm. per kilo to about 0.130 gm. and given one hour before, raised the tolerance to about 0.140 gm. per kilo.

(*c*) The maximum tolerated dose of a solution of the disodium salt of a lot of arsphenamin was 0.130 gm. per kilo; when administered twenty four hours after the preliminary intravenous injection of 0.005 gm. per kilo the maximum tolerated dose was more than 0.150 gm. per kilo. With another toxic lot of arsphenamin borne only in dose of 0.090 gm. per kilo, the preliminary injection of 0.005 gm. per kilo twenty-four hours before raised the tolerance to 0.100 gm. per kilo. With a second toxic lot borne only in dose of 0.090 gm. per kilo, the preliminary injection of 0.005 gm. per kilo twenty-four hours before raised the tolerance to 0.120 gm. per kilo.

(*d*) With neoarsphenamin the results were not as definite and in most experiments, the preliminary injections did not increase the tolerance; indeed, the reverse was frequently noted due, in part, to excessive hemorrhages, since neoarsphenamin especially increases the bleeding time. In one experiment, however, the preliminary injection of 0.005 gm. of neoarsphenamin per kilo one minute and one hour before apparently raised the tolerance for a particular lot from 0.280 gm. to 0.320 gm. per kilo.

It would appear, therefore, that the preliminary injection of a small dose of arsphenamin or neoarsphenamin may materially increases tolerance for these drugs and especially for arsphenamin. The method doubtless possesses practical clinical value as shown by Danysz, Stokes and Busman, but it is to be borne in mind that the use of preliminary injections of atropin, adrenalin or of the drug or of a combination of $\frac{1}{10}$th of the dose of drug intravenously and $\frac{1}{50}$th grain of atropin subcutaneously about one hour before the main injection, are only indicated for those occasional individuals who are hypersensitive to the drugs and likely to develop the immediate vasoparetic reaction. These methods are not particularly advised for the prophylaxis of the gastro-intestinal, protein shock or late reaction as dermatitis and

jaundice, although they may be indicated in cases of several gastro-intestinal reactors since these are to be interpreted as acute vasoparetic reactions of the gastro-intestinal mucosa.

Contra-indications to the Administration of Arsphenamin, Neoarsphenamin and Other Organic Arsenical Compounds.—This naturally brings up for consideration the question of the contraindications to the administration of the organic arsenical compounds in the treatment of syphilis and other diseases. Rather formidable lists of various acute and chronic diseases have been published by different writers, but as our knowledge of the pharmacology and toxicology of arsphenamin and its substitutes has advanced the list of contraindications has diminished. Furthermore, while one drug of the group, as arsphenamin, for example, may be contraindicated in any given individual, another as neoarsphenamin or sulpharsphenamin, may be given with safety.

Probably the only real contraindication to these drugs as a group is *hypersensitiveness to arsenic.* Individuals developing acute reactions, dermatitis and allied lesions, jaundice, etc., are always bad risks and usually demand that treatment shall be continued with other antisyphilitic drugs as mercury and bismuth. It is sometimes possible, as in cases developing minor *dermatoses,* to continue arsenical treatment with another kind of compound beginning with small doses, gradually increased, but with a sharp lookout for urticaria, fleeting erythemas, itching of the palms and soles, paresthesia, etc. Sometimes individuals developing acute reactions after intravenous injections of arsphenamin or neoarsphenamin can stand small, ascending doses of sulpharsphenamin by subcutaneous or intramuscular injections; cases of dermatitis due to sulpharsphenamin may sometimes bear intravenous injections of neoarsphenamin, but I repeat, on the bases of experience, that extraordinary caution is required when arsenical treatment is resumed after the subsidence of any of the different arsenical dermatoses described in Chapter LI.

Cases of *jaundice* are always bad risks unless they are due to syphilitic hepatitis when just the reverse is true, But if jaundice has been produced by the previous injection of arsenical compounds, there are risks of its recurrence from the administration of any of the organic arsenicals although arsphenamin is probably better borne by these cases than neoarsphenamin. Likewise in cases of jaundice due to infection of the gall-bladder and ducts it is possible for functional activity to be so reduced as to lower the tolerance of the organ for arsenic and due caution must be exercised with preference for small doses in order to avoid too sudden accumulation of arsenic with possible histological changes of cloudy swelling and fatty degeneration.

Conditions of these kinds, may, therefore, absolutely forbid the administration of arsenic in any form. It is true that very small doses may be given even under such circumstances, but the question then arises whether they would be worth while from the standpoint of therapeutic results. In a broad and general manner, I think it may be stated that, whenever doses of 0.1 to 0.2 gm. are contra-indicated, it is better to abandon arsenical treatment and employ mercury or potassium tartrobismuthate in conjunction with the iodids. This is especially true in the presence of acute syphilitic lesions; it is not necessarily true, however, in chronic syphilis since doses of 0.1 gm. of arsphenamin or neoarsphenamin or even less over long periods of time sometimes result in surprisingly good results and especially in chronic cardiovascular syphilis.

Arsphenamin is sometimes contra-indicated in *grave cardiovascular disease* with high blood-pressure, because of the dangers attending a sudden increase

of blood-pressure due to the drug, the injection of 200 c.c. or more of solution, fear, or a combination of these. In advanced syphilitic aortitis and myocarditis and especially if meningovascular syphilis is suspected or known to be present, preference should be given to those arsenicals like neoarsphenamin or sulpharsphenamin, that may be given in concentrated solution with a syringe with the minimum of excitement. Cases of syphilis with intracerebral hemorrhage (apoplexy) especially require great care although small (0.2 gm. or less) doses of neoarsphenamin may be given with safety insofar as increasing blood-pressure is concerned. The same is true in case of *tuberculosis with hemoptysis;* the arsenicals are not actually contra-indicated, but due care must be exercised in their administration with special reference to the use of small doses of those drugs like neoarsphenamin, that may be given in concentrated solutions with least chances of suddenly increasing blood pressure. Objection may be raised to neoarsphenamin because it tends to lower the coagulation and bleeding time of the blood, but in human beings these effects are not noted with the doses ordinarily employed.

Other diseases as *advanced diabetes and non-syphilitic nephritis* are usually mentioned as contraindications. This may be true insofar as full sized doses are concerned, but not with smaller doses if hypersensitiveness to arsenic is absent. Diabetes mellitus is not itself a syphilitic disease although the diabetic may have syphilis in addition and the latter may require treatment. Doubtless, the tolerance of advanced cases is much lower than among non-diabetic individuals, but the difficulty is usually solved by the administration of neoarsphenamin in doses of 0.2 to 0.6 gm. Advanced nephritis is of course a grave danger because of the possibility of interference with the elimination of arsenic. Syphilitic nephritis usually improves rapidly under arsenical treatment and I have observed the rapid disappearance of albumin and casts in the urine in cases of non-syphilitic nephritis under neoarsphenamin treatment. Certainly arsphenamin or neoarsphenamin is to be preferred to either mercury or bismuth and, if treatment must be given in spite of the nephritis, I believe that intravenous injections of neoarsphenamin in dose of 0.2 to 0.4 gm. once a week or intramuscular injections of the same amounts of sulpharsphenamin are the remedies of choice.

It is sometimes stated that the arsenicals should not be given to persons of *advanced years*. Age as such is not an absolute contra-indication, but the possibility of stirring up latent syphilitic infection in the nature of Herxheimer reactions may be worthy of very serious consideration. When chronic latent syphilis is discovered in the aged, it is sometimes better to leave the disease untreated; acquired acute syphilis of the aged of course requires treatment. At all events, the kidneys and liver of those over 65 years of age are not apt to be as functionally active as in younger years and our method of treating individuals of this age or older should be along less energetic lines than is possible with younger persons. Arsphenamin and its substitutes, therefore, are not necessarily contra-indicated in those of advanced years; only full-sized doses and intensive methods of treatment may be contra-indicated with these compounds as is likewise true of mercury and bismuth. Furthermore, clinical judgement must be exercised in deciding whether or not treatment is indicated at all. It is sometimes a matter of wisdom not to treat cases of chronic latent syphilis, although in the presence of chronic active syphilis with progression of lesions and symptoms enough treatment must be usually given to hold the disease in check. But, whenever treatment is given, it should begin mildly and with small doses to minimize the danger of Herxheimer reactions and especially if the nervous system is involved.

In *syphilis of the optic nerve* or other portions of the eye, pentavalent arsenicals as atoxyl and tryparsamid may be contra-indicated, because of the well-known tendency of these compounds to produce toxic amblyopias. The trivalent compounds as arsphenamin, neoarsphenamin and sulpharsphenamin, however, may be given but always in small, ascending doses in order not to produce Herxheimer reactions. The same is true in syphilis of other of the cranial nerves and especially the auditory and oculomotor nerves in order to guard against the production of neural Herxheimer reactions (neuro-recidives).

In severe endocrine disturbances and especially of the thyroid and adrenal glands, the arsenicals should not be given or if so, in small, guarded doses. Likewise in *pregnancy*, the arsenicals should be administered with all precautions against severe reactions because of the danger of producing abortion or miscarriage; my own preference is for neoarsphenamin. It need scarcely be stated that in *acute infectious diseases* or immediately thereafter, tolerance for these compounds may be reduced indicating smaller doses than usual although the arsenicals are not absolutely contraindicated.

As previously stated, therefore, the only absolute contra-indication to the administration of arsphenamin and its substitutes is hypersensitiveness to these compounds rendering dangerous or impossible the administration of even small doses. Fortunately, the number of such cases is indeed very small. On the other hand there are some diseases in which the doses of the various arsenicals by intravenous injection must be reduced or preference given subcutaneous or intramuscular injections of sulpharsphenamin in the interests of safety and in which one kind of arsenical may be less dangerous and to be preferred to others.

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CHAPTER LI

ETIOLOGY, SYMPTOMS AND TREATMENT OF TOXIC REACTIONS FOLLOWING THE ADMINISTRATION OF ARSPHENAMIN, NEOARSPHENAMIN AND OTHER ORGANIC ARSENICAL COMPOUNDS

Having thus reviewed in the preceding chapters the general physico-chemical and chemical causes of toxic reactions produced by the administration of some of the medicinal organic arsenical compounds, as well as those reactions more properly ascribed to pathological conditions of individual

patients or to methods of administration and the prevention of these reactions as based upon our present knowledge of these many and diverse causes, we may now consider from the standpoint of clinical manifestations and treatment the different symptom-complexes and lesions and especially those sometimes produced by the intravenous injection of arsphenamin, neoarsphenamin and sulpharsphenamin. From the etiological standpoint the subject is still quite obscure and in most instances the toxic reactions cannot be ascribed exclusively to a single sharply defined agent, but are due rather to a combination of causes in which one, perhaps, may stand out with particular prominence and importance.

Usual Effects Produced by the Administration of Arsphenamin and Its Substitutes.—However, before discussing the toxicological reactions sometimes produced by the *intravenous* injection of arsphenamin and its substitutes a few words are required concerning what may be called the normal or physiological effects, because minor and unimportant reactions occur with some individuals which cannot properly be designated as truly toxicological. Furthermore, some patients are very nervous and dread the injections which alone may be responsible for signs and symptoms of syncope and nausea without in any way incriminating the drug or method of administration as reported by Bender[1] and others; indeed, such symptoms may develop with the introduction of the needle and before any drug has been injected at all due entirely to fear and nervousness and especially if much pain has been produced.

Furthermore, it is to be emphasized that in the great majority of cases the intravenous injection of neoarsphenamin and supharsphenamin by technically correct methods is not followed by any untoward effects whatsoever; arsphenamin is more likely to produce minor disturbances due to the greater variation in physicochemical properties as well as to the injection of larger volumes of fluid, but since 1920 the incidence of toxic reactions produced by arsphenamin and neoarsphenamin has certainly decreased in comparison with the incidence of reactions in preceding years.

Probably the first effect to be mentioned by the patient is a peculiar *odor* and *metallic taste,* usually resembling ether; this very commonly happens after the intravenous injection of the first few cubic centimeters of solutions of arsphenamin and neoarsphenamin and in no way indicates a toxicological reaction of the approach of such. Evidently this effect is due to a circulation of the drug through the nose, mouth and accessory glands with rapid elimination and indicates the extremely rapid diffusion of these drugs administered by intravenous injection. It is my custom to advise patients to chew a chiclet or some other highly flavored gum for fifteen minutes during and after the injection, which aids in disguising the ether taste.

Not infrequently patients experience a *feeling of fullness* in the head and show slight *flushing of the face* which last for only a few minutes or indeed seconds, due to the physiological effects of arsphenamin upon the vasomotor system, and, in the case of arsphenamin, to the increase of blood-pressure and the injection of 100 c.c. or more of fluid. These effects may also occur after the injection of neoarsphenamin and sulpharsphenamin, but only occasionally and in exceptional cases, since these compounds do not affect the vasomotor system to the same degree as arsphenamin and are usually administered in concentrated solutions. The physician may note the flushing followed by slight *perspiration,* without any complaint from the patient. Sometimes these fleeting effects are followed by a slight feeling of *faintness* which quickly passes off, to be followed by a sense of elation that the ordeal of injection is over. Sometimes nausea and even vomiting occur

and especially if the patient is nervous and has eaten within half an hour of the time of injection, but these effects quickly pass away and are due primarily to the ether taste which is especially disagreeable to patients who have taken ether for anesthetic purposes on former occasions.

In some cases of chronic latent syphilis the first injection or two of arsphenamin or neoarsphenamin is followed within a day or two by malaise, some loss of appetite and vague pains in the heart, lymph-glands and muscles. I have interpreted these pains as due to the provocation of latent areas of syphilitic infection which are not in a strict sense the results of toxicological effects of the drugs and which have been discussed in Chapter XLVIII with more detail.

Intramuscular and subcutaneous injections of sulpharsphenamin usually produce brief burning and stinging pain but rarely nausea and vomiting. I have already discussed these local effects in Chapter XLIX; here it may be emphasized, however, that children as a rule complain much less than adults and adults vary greatly in their reactions to intramuscular injections. Some refuse injections by this route after the first on account of the pain and disability produced and this is not always due to imagination or nervousness but to a reduced state of general nutrition and resistance which results in excessive local reactions.

Acute Physico-hemoclastic Reaction, with Special Reference to That Produced by Acid Solutions of Arsphenamin.—*Etiology.*—Under this heading I refer to the acute and frequently fatal reactions caused by the intravenous injection of solutions of arsphenamin in water without neutralization (acid solutions); as stated in Chapter XLV, the injection of such solutions and particularly the rapid injection of concentrated solutions, may produce profound intravascular reactions of agglutination, hemolysis and precipitation with severe and sometimes fatal effects. I have known of several fatalities of this kind due to errors on the part of physicians injecting concentrated solutions of arsphenamin in mistake for neoarsphenamin and sulpharsphenamin. If acid solutions of arsphenamin are well diluted as 0.4 to 0.6 gm. dissolved in 200 to 300 c.c. of water, and slowly injected, the patient may escape a severe reaction and, indeed, not a few individuals owe their lives to these fortuitous circumstances.

Severe reactions of this kind due primarily to acute physical intravascular reactions have also been caused by solutions of arsphenamin to which too much sodium hydroxid has been added; also by solutions to which sodium hydroxid has been added followed by the addition of an excess of hydrochloric acid when the physician has been in doubt regarding the end reaction and fitness of the solution. Whether or not the acute immediate reactions produced by the intravenous injections of properly neutralized (monosodium salt) or slightly alkalinized (disodium salt) solutions of arsphenamin and cloudy solutions of neoarsphenamin are to be placed in this category, cannot be stated. Doubtless they are in part due to physicochemical phenomena as changes in the viscosity, solubility, etc., of the solutions and in some cases it is possible that intravascular agglutination and precipitation may occur; but as stated in Chapter XLV, the plasma affords a great degree of protection against these changes when ordinary doses are given whereas with the rapid injection of acid solutions of arsphenamin this protection is generally insufficient. In other words, intravascular reactions may play a part in the production of the "nitritoid crisis" or acute vasoparetic reaction shortly to be discussed, but under the present heading I include those reactions in which intravascular physical changes of hemolysis, agglutination and precipitation are primarily or solely responsible for the toxic effects and especially

those due to errors in administration, as the intravenous injection of concentrated acid or over alkalinized solutions of arsphenamin.

Symptoms.—The symptoms develop almost immediately and are mainly due to widespread embolism and infarction, especially of the lungs, with secondary cardiac dilatation. The patient expresses a fear of approaching death, presents an anxious and appealing expression, develops hiccough or short spasmodic cough and complains of severe pain in the thorax and especially a sense of constriction and suffocation. The pulse is rapid and at first full and bounding followed by irregularity and extreme weakness; coma generally follows from cerebral anemia, although convulsions may be present apparently due to cerebral embolism involving motor areas. In not a few cases death has followed immediately or within an hour or two or the patient partially recovers to succumb during the following twenty-four hours. In less severe cases when the injections have been interrupted in time, recovery from the acute reaction has occurred followed by the development of bronchopneumonia due to embolism. In not a few instances the physician has discovered his error of failure to neutralize or alkalinize the solution almost immediately after infusion of the solution has been started and immediate interruption of the injection has been followed by no untoward effects from the injection of a few cubic centimeters; under such circumstances there is nothing to fear, toxic reactions are not likely to develop and treatment is unnecessary except possibly the intramuscular injection of atropin to stimulate the heart.

Treatment.—The first step in treatment is, of course, to interrupt the injection upon the first signs of distress. If, however, the whole dose has been given by rapid intravenous injection with a syringe there is little that may be done. The patient should be laid flat and if pulseless or nearly so adrenalin should be injected *intravenously* in dose of 0.1 c.c. (1 to 2 minims) of 1:1000 solution diluted with water for the purpose of stimulating the heart and especially the right ventricle. For less severe reactions it may suffice to inject the adrenalin solution intramuscularly in dose of 0.5 to 1 c.c. (8 to 15 minims). In either case it may be necessary to prolong the stimulation by subsequent intramuscular injections. Subcutaneous injections are much less efficacious and oral administrations are useless.

Jackson and Smith [1a] have suggested intravenous injections of tyramin (parahydroxyphenylethylamin) in dose of approximately 0.001 gm. per kilogram of weight which, for an adult patient of average size, would be about 0.06 gm. or 1 grain. Tyramin stimulates the heart and produces a more prolonged and sustained use in systemic blood-pressure than adrenalin and may thereby prove useful for the treatment of collapse; I have had no personal experience with the drug, however, and under ordinary circumstances it is not as readily available as adrenalin.

At the same time atropin sulphate should be injected intramuscularly in dose of $\frac{1}{50}$th grain for the purpose of stimulating the circulation, relaxing the bronchi and possibly accelerating respiration by stimulation of the center and increased formation of carbon dioxid. If convulsions are present, atropin must of course be administered with great care in order not to overstimulate the motor areas and other higher divisions of the neural axis. Caffein and camphor are likewise efficacious by subcutaneous injection as heart stimulants and the primary object of treatment is to maintain cardiac and respiratory functions.

If the patent survives the acute shock, the purpose of treatment thereafter is to combat the effects of embolism and especially of the lungs. For this purpose he should be placed in bed, surrounded with hot water bottles if

the temperature is subnormal or a chill supervenes and sedatives administered especially if there is pain in the chest with coughing. For these purposes codein may be given, although small doses of morphin by subcutaneous injection are usually to be preferred. Counter irritation to the chest, front and back, is sometimes useful and especially dry cupping or hot turpentine stupes, the latter repeated at intervals.

Acute Vasoparetic Reaction or "Nitritoid Crisis."—One of the best known and most frequently mentioned of the toxic reactions sometimes following the intravenous injection of arsphenamin, neoarsphenamin and their substitutes, is the "nitritoid crisis," so designated by Milian[2] in 1912 because the lesions and symptoms resemble those produced by the inhalation of amyl nitrite. The phrase has since come into very general use and is frequently employed to cover any and all of the acute untoward reactions following the injection of the organic arsenical compounds. As a matter of fact, however, the reaction may be produced by the intravenous injection of various substances and as we will shortly discuss under etiology, may be caused by a variety of seemingly unrelated agents. The outstanding change is one of acute congestion apparently due to loss of vasomotor control, a condition of vasoparesis, and the term "vasoparetic reaction" would appear to be especially appropriate.

The *frequency* of these reactions is difficult to state and has varied greatly in the experience of different syphilologists. Undoubtedly they were more frequent prior to 1920 than at the present time and are apparently produced by injections of arsphenamin more frequently than by neoarsphenamin and sulpharsphenamin. Different lots or batches of arsphenamin and neoarsphenamin have been known to produce more of these reactions than other lots and the arsphenamins prepared by different laboratories vary in this regard. In 1912, Milian observed as high as 4.8 per cent. "nitritoid" reactions; in 1917 Jones and Gibson[3] found the incidence approximately 0.5 per cent. and I believe it may be stated at present that they occur in less than 1 per cent. of injections. Acute or immediate reactions of this kind are not ordinarily observed following intramuscular injections of arsphenamin or its substitutes and especially sulpharsphenamin, although such delayed reactions as nausea and vomiting and the acute central nervous system vasoparetic reaction developing a few days after injection have been produced by this route of administration. Dennie[1] states that he has never seen the reaction after ten thousand doses given to children over a period of four years, but it is not stated whether most of these injections were by the intravenous or intramuscular routes and, as just mentioned, the incidence is very much higher after intravenous than after intramuscular or subcutaneous injections.

Etiology.—The subject of the etiology of these reactions is still quite obscure; we know in fairly definite terms of several causes but little or nothing of the exact mechanism of their action. The outstanding and prominent lesion is one of acute vasodilatation with serous exudation and this change may be brought about by seemingly unrelated agents.

Milian has stated that the reaction was due to acute adrenalin insufficiency and Berward,[5] Hirano[6] and others have subscribed to this view. It is true that the injection of adrenalin may give prompt relief from the symptoms or prevent the reaction if administered prior to the injection of the arsphenamins by its well known vasoconstrictor effects, but this in itself is not proof that the reaction is caused by a sudden diminution of adrenalin in the blood presumably by an effect of the arsenical upon the suprarenal glands directly or through the sympathetic nervous system. Hirano studied the adrenalin store of rabbits' adrenals after intravenous injection of arsphenamin and neoarsphenamin employing the colorimetric method of Comessatti and also investigated a number of glands histologically. He concluded that large doses of these arsenicals produced a remarkable decrease of chromaffine substance, and of residual adrenalin and that in therapeutic doses arsphen-

amin caused partial exhaustion, while neoarsphenamin did not influence the adrenalin contents.

As stated in Chapter XLVI, Lucke and I observed histological changes in the adrenal glands of rabbits and rats injected with very large doses of arsphenamin and neoarsphenamin similar to those previously described by Pearce and Brown, but the changes produced by amounts corresponding to the doses ordinarily administered to human beings according to body weight, were almost without significance. Lucke, McCouch and I[7] then studied the subject along the lines of Hirano's investigations, but likewise with negative results. We injected rabbits intravenously with amounts of disodium arsphenamin ranging from 0.02 to 0.05 gm. and with neoarsphenamin in doses of 0.1 gm., per kilogram of weight which were therefore from two to five times the amounts ordinarily given human subjects at one time and at intervals of fifteen minutes to eighteen hours later extracted the right adrenal glands by the method of Folin, Dennis, and Cannon[8] and assayed them by the biological method of Elliott.[9] A considerable range of adrenalin values was obtained, but on the average, the amounts found were almost identical with the average of the adrenalin content of the glands of untreated control rabbits. In a second series of rabbits injected intravenously with two to twelve doses of arsphenamin and neoarsphenamin in amounts corresponding to the doses for human subjects according to body weight, the adrenalin content of the glands was practically unchanged and when examined histologically did not show any noticeable or constant alterations of the chromaffin reaction. The lipoids appeared to be very slightly increased after single large injections and slightly decreased after multiple injections.

These experiments, of course, do not exclude the possibility of arsphenamin and its substitutes producing acute temporary decrease of adrenalin in human subjects either by direct action upon the glands or through the sympathetic nervous system; they do indicate, however, that this action is improbable and at least show quite clearly that the glands and their adrenalin store are not constantly or perceptibly changed insofar as such may be studied experimentally. Recently Levy and Juster[10] have drawn attention to the frequency of "nitritoid" reactions among individuals with symptoms of adrenal insufficiency as apathy and asthenia; also among those with thyroid disturbances and they suggest that the "nitritoid crisis" may be due to disturbance of some of the endocrine glands. Juster[11] has especially emphasized the possibility of a simultaneous parasympathetic stimulation and sympathetic paralysis as a cause of these reactions and believes that injections of adrenalin afford relief by stimulating the sympathetic nervous system. He has observed the reaction more particularly among those in a vagotonic state and has re-named the symptom-complex the "vagotonic crisis." Flandin, Tzanck and Roberti[12] and other French syphilologists have likewise subscribed to the view that the "nitritoid crisis" is due to some disturbance of the sympathetic nervous system which in turn produces disturbances of certain of the endocrine glands including the adrenals. Rosen, Müller and Myers,[12a] observing that the intravenous injection of arsphenamin is followed by a decrease of leukocytes in the peripheral blood, believe that this effect and the influence of adrenalin upon the angioneurotic symptoms indicate that the drug influences the autonomic nervous system rendered hypersensitive by some constitutional factor or by syphilitic infection.

I think it is to be granted that the lesions and symptoms of the "nitritoid crisis" are due to a disturbance of the sympathetic nervous system insofar as the vasomotor nerves are concerned. This disturbance is characterized by immediate or almost immediate vasomotor paralysis which may be general or more or less localized. This effect upon the sympathetic nervous system is, however, a direct and primary one without involving in any essential manner the endocrine glands although it is to be granted that disturbances of these glands and more especially of the suprarenals, may render the sympathetic nervous system more susceptible to the toxic effects of the organic

arsenicals and other unrelated agents known to produce the vasoparetic reaction.

What produces this vasoparesis? Is it due to a toxic impurity in the drug? Is it due to a state of natural or acquired allergy to arsenic? Is it due to physicochemical factors involving the colloidal chemistry and interaction between solutions of the medicaments and the plasma of the patient? Before attempting to discuss these possibilities, it is clear that any explanation of the mechanism must satisfactorily meet the following two clinical facts in order to be acceptable: first, that some lots of arsphenamin and neoarsphenamin have produced the reactions when other lots prepared by the same laboratory did not and that chemical analyses and rat toxicity tests have generally failed to show any essential differences among them or to disclose the toxic agency in the former; secondly, that some individuals have developed the reaction when injected with the same compound in exactly the same manner as numerous others who escaped reactions. The question of etiology now resolves itself into what constitutes the toxic agency and why human beings vary in susceptibility to it.

In my opinion, the acute vasoparetic reaction or "nitritoid crisis" may be due either to toxicity of the medicament or to an abnormal change in the capillaries of the individual. In case of the former, reactions of this kind may be produced in human beings with a normal vasomotor system; in the case of the latter reactions may be produced in individuals with an abnormal vasomotor system by compounds perfectly well borne by the great majority of human subjects. In still a third group the reactions may be due to a combination of these two factors, namely, an abnormal vasomotor system and the injection of a compound containing arsenic in such a physical colloidal state as intensifies the physiological vasodilating effects of arsenic. My reasons for this opinion are as follows: The physiological action of arsenic is to produce paralysis of the capillaries with increased permeability and especially of the vessels of the splanchnic area. This has been known for many years, is a well-established fact in the pharmacology of arsenic and in 1912 Ricker and Knape[13] showed by direct microscopic observations of the capillary beds in living animals during the administration of arsphenamin and neoarsphenamin that both drugs caused a slowing of the blood stream, dilatation of the capillaries, stasis and hemorrhage. At the same time the heart is involved by a similar action of the arsenic upon the nerve ganglia and the pronounced and persistent fall in blood-pressure is due to cardiac failure and arterial vasomotor paralysis or depression, aided probably secondarily to some extent by diminution of blood volume by transudation of plasma due to increased capillary permeability.

Under normal or usual conditions this effect of arsenic following the intravenous injection of arsphenamin and its substitutes is not apparent or is so mild and fleeting as to produce but mild and temporary effects. But in some individuals with instability of the vasomotor system due to endocrine disturbances or factors of unknown nature, these physiological effects are greatly exaggerated resulting in the production of extreme vasoparesis or the extreme sensitiveness of the vasomotor nerves to the physiological effects of arsenic may be due in some cases to accumulation of arsenic in the tissues or to congenital or acquired hypersensitiveness to arsenic. As stated in Chapter XLVII, there can be little or no doubt that some individuals are naturally hypersensitive to arsenic just as others may be to quinin, cocain and other drugs; also that individuals receiving numerous injections of arsphenamin or one of its substitutes may develop an intolerance for the drug, either as a result of accumulative toxic effects or of the acquisition of

allergic hypersensitiveness. The results would be the same in either case, because cumulative toxic effects of arsenic would involve the nerves of the capillaries and allergy in man is characterized by vasomotor paralysis due either to a direct effect upon smooth muscle in general including that of the blood vessels or upon the vasomotor nervous system. In other words, the reasons why some individuals develop acute vasoparesis following injections of arsphenamin or one of its substitutes which produce no untoward effects in other individuals may be due to any one or a combination of these changes in the individuals themselves and not to the drug: (*a*) Because their sympathetic vasomotor nervous system is unstable by reason of endocrine or other disturbances resulting in increased susceptibility to the physiological vasoparetic effects of arsenic; (*b*) because arsenic has accumulated from previous administrations and produced a state of vasomotor instability or (*c*) because the individual has a natural congenital or acquired state of hypersensitiveness to arsenic which results in acute vasomotor paresis, either because of allergic sensitiveness of the smooth muscle of the capillaries or an effect upon the vasomotor nerves. Individual susceptibility may be due, therefore, to any one or several of three unrelated factors involving the capillaries direct or their nerves.

But why are some lots of arsphenamin or neoarsphenamin more likely to produce this reaction than others? As previously stated, some batches of arsphenamin have produced so many reactions of this kind that they could not be employed. What is even more mysterious is that other physicians have used the same lots with absolutely no untoward reactions. Chemical analysis and rigid animal tests of such lots have repeatedly shown in my experience no changes from the usual and average, although these do not exclude the possibility that impurities were present. In my opinion, the effects are due to arsenic either from the molecule of the drug or from an arsenical impurity. In other words, the mechanism of its toxic action would appear to be referable more to physical (colloidal?) changes affecting the vasomotor system than to chemical injury of the capillaries. But just what may be the exact nature of these physical changes I am unable to state. As reviewed in Chapters XLV and XLVIII, it is apparent that the concentration of the solution, its age before injection and rapidity of injection, the temperature of the water or saline solution employed for preparing solutions, the degree of shaking necessary to effect solution and, above all, the reaction of the solution, are factors of varying importance influencing and modifying the viscosity and probably other physical colloidal properties of the solutions and particularly solutions of arsphenamin. In my opinion, these factors influence primarily the physiological action of the arsenic. In some lots of arsphenamin the change may be due to manufacture and thereby explain vasoparetic reactions with a given lot in the hands of different physicians; in other and more numerous instances the changes may be brought about during the preparation of solutions and thereby explain why a given lot produces reactions in the hands of some physicians and not in the hands of others. I do not know the exact nature of these changes in the physical (colloidal?) chemistry of the arsenic of arsphenamin and its substitutes; neither do I know how it produces vasoparesis other than it appears to intensify the physiological vasoparetic effects of arsenic. I very much doubt if the reactions are due to intravascular agglutination, hemolysis or precipitation such as occurs when concentrated acid solutions of arsphenamin are injected intravenously, although, as previously described, the symptoms may be similar insofar as fall of blood pressure and pulmonary symptoms are concerned; the plasma is probably able to prevent the slight agglutinating

and hemolytic effects of dilute solutions of disodium arsphenamin slowly injected and there are no evidences at all of intravascular reactions of this kind from the injection of isotonic solutions of neoarsphenamin although this arsenical may produce the "nitritoid crisis."

In the final analysis, this acute toxic reaction may be due, therefore, primarily to an exaggeration of the physiological effects of arsenic of arsphenamin and its substitutes. In the majority of cases no ill effects are noted. In some, however, toxic reactions are produced either because the capillaries and the vasomotor nerves are unusually sensitive to the vasoparetic effects of arsenic or because the arsenical compounds undergo some change in the course of manufacture and in the preparation of the solutions as results in increasing the toxicity of the arsenic or in such physical (colloidal?) changes as greatly exaggerates its physiological effects upon capillaries.

Symptoms.—The symptoms are due directly or indirectly to the acute vasoparesis and may be of a most varied character according to the capillary beds involved and the degree of dilatation and exudation. As a general rule, the patient first complains of a peculiar metallic taste resembling ether, garlic, vinegar, etc. and of feeling queer with a sense of "fullness" of the head, pain in the gums and teeth, nausea and a burning sensation of the skin of the face and head and particularly of the lips and tongue; sometimes there is immediate vomiting. The face is flushed, particularly the cheeks and ears, and is accompanied by injection of the conjunctivae and eyelids with lachrymation and dilatation of the pupils, the eye symptoms being especially characteristic. Not infrequently the only symptoms are syncope followed by headache and nausea or immediate flushing and burning pain of the face, passing of the fingers through the hair, nausea and vomiting; in mild cases and especially if the injection has been slowly given and promptly interrupted, no other symptoms develop, leaving the patient badly frightened and a little weak but otherwise well.

In more severe cases, however, the tongue and lips become very much swollen and dusky red in color with husky voice and coughing due to swelling of the mucous membrane of the oral and respiratory tracts. The pulse at first rapid and bounding may become weak and rapid with marked pallor of the skin and profuse perspiration; retching and vomiting; loss of control of the sphincters, shallow respiration, coughing and backache may be present. The patient feels that he is going to die, certainly looks as if he may but rarely does. In some cases consciousness is lost for a brief time and convulsive twitchings may develop. Sometimes the skin and especially that of the chest and neck develops a "goose skin" appearance, the elevations gradually turning pink and developing into an urticarial rash which may be localized to the areas mentioned or involve other portions of the body.

As previously stated, these lesions and symptoms may be explained entirely by the sudden dilatation of the blood-vessels and especially of the capillaries; why the capillaries of the conjunctivae and skin of the face generally are primarily involved cannot be stated. Bargues[14] and others have described cases of localized "nitritoid crises" involving the arm and certain nerves following injections of neoarsphenamin. The capillary dilatation with serous exudation is likewise responsible for the swelling of the lips and tongue, the urticarial eruption and possibly of the dyspnea and cough by involvement of the bronchial mucosa. The succeeding pallor, rapid and weak pulse, perspiration and coma are probably due to splanchnic dilatation and cardiac depression and dilatation. Vomiting has been explained on the basis of congestive irritation of the vomiting centre or it may be due to the general meningeal and cerebral congestion evidently responsible for the feel-

ing of "fullness of the head" of which most patients complain. As reported by Keidel and Kemp[14a] cases of cardiac syphilis (aortic insufficiency and myocarditis) may especially develop alarming symptoms of sudden syncope with a greenish pallor, cold sweats, gasping respiration, bradycardia, reduction of blood pressure, etc., evidently associated with acute cardiac damage.

Hemorrhagic Encephalitis (Acute Cerebromeningeal Congestion and Edema). In rare instances, the cerebral and meningeal congestion produces more alarming and dangerous symptoms coming on two to four days later and constituting what Milian has designated as "*serous apoplexy;*" this condition is characterized by extreme congestion of the cerebral meninges, serous exudation with greatly increased cerebrospinal fluid pressure and in some instances, with minute hemorrhages in the meninges and substance of the brain. In German literature the syndrome is usually designated as "*hemorrhagic encephalitis.*"

In mild cases there may be only severe headache, fever and vomiting which pass off without special treatment. As a general rule the patient exhibits some signs of a "nitritoid crisis" at the time of injection, but recovers and all goes well until the third or fourth day when cerebral symptoms suddenly develop. As described by Sanphar,[15] Castello[16] Dennie,[17] Fraser and Duncan[18] and others, the patient becomes extremely restless and apprehensive followed by severe headache, edema of the face, vomiting and dyspnea; thereafter the patient becomes unconscious with signs of severe cortical irritation as clenched teeth, epileptiform convulsions or general twitchng, cyanosis, rapid and stertorous breathing followed later by shallow, irregular breathing as the respiratory center becomes exhausted. Coma, the Cheyne-Stokes syndrome, incontinence of urine and feces develop and death may follow in twenty-four to forty-eight hours.

In these cases, the spinal fluid shows the changes of "meningismus" or so-called "serous meningitis" being under increased pressure, normal in gross appearance but otherwise showing the pathological changes of syphilis.

Necropsy examination is likely to reveal an acute hyperemia of the meninges with disseminated punctate hemorrhages. Capillary hemorrhages may also be found in the liver and kidneys and the lungs may likewise show acute congestion with interstitial hemorrhages.

Ehrlich believed that these lesions and symptoms were produced by oxidation products and particularly by "arsenoxid" producing adrenal insufficiency. McDonagh believes that they may be due to toxins liberated from spirochetes and paralyzing the vaso-constrictor nerves. Dudley[19] believes that the most important cause is an insufficient supply of oxygen to the tissues—an anoxemia. Hitch[20] has also subscribed to this view believing that the widespread capillary hemorrhages afford a clear anatomical basis for the hypothesis and occurring in the lungs are responsible for the anoxemic state.

Treatment of the "Nitritoid Crisis."—The first step in treatment is, of course, to interrupt the injection if all of the solution has not been given. *The patient should then be laid flat* and the clothing loosened. A drahm or two of aromatic spirits of ammonia diluted with water should be given and in mild cases nothing more may be required. As a general rule, however, more treatment is indicated and since Milian first suggested *intramuscular or subcutaneous injections of adrenalin*, numerous reports have shown that this is the remedy of choice, as stated by Ravaut,[21] Darier[22] and other French syphilologists, as well as by Pollitzer,[23] Berman,[24] Meddis and Sterling[25] Beeson[26] and numerous other American syphilologists. Adrenalin in adequate dosage produces a constriction of the blood-vessels and especially of

the splanchnic area by stimulating the myoneural junctions of the postganglionic fibers of the sympathetic nerves and also stimulates the heart by action upon the terminations of the accelerator nerves in the muscle of this organ. It effects, however, are fleeting and several injections may be required but due care must be exercised against over dosage to avoid producing "delirium cordis."

Pituitrin may be also of some value and may be used along with adrenalin. It tends at first to produce a slight fall in blood-pressure by cardiac depression along with a slowing of the heart beats due to reflex vagus stimulation, but these effects are followed by a rise in pressure due to vasoconstriction through direct stimulation of the smooth muscle of the blood-vessels; Milian states that a combination of adrenalin and pituitrin (hypophysine) is good, since the effects of the latter are more prolonged. The dose is 1 c.c. (15 minims) of the liquor hypophysis (pituitary solution) by subcutaneous injection.

Atropin sulphate in dose of $\frac{1}{50}$th grain by subcutaneous injection has been especially advocated by Stokes[27] and Busman[28] and is particularly valuable for the prophylaxis of these reactions as has been already discussed in the preceding chapter. Atropin tends to relax the bronchi and thereby relieve the dyspnea and air hunger due to the acute congestion of the mucosa; in the dose advised it is also possible that the respiratory centre is stimulated with acceleration of the breathing rate and depth. Hanzlik and Karsner[29] state that atropin relieves the pulmonary symptoms by improvement in the circulation rather than by any effect upon the smooth muscle of the bronchioles.

Jackson and Smith[30] have found *tyramin* especially useful in the treatment of dogs severely poisoned by arsphenamin, observing that in dose of 0.001 gm. per kilo (corresponding to 0.06 gm. or 1 grain for an adult of average size) by subcutaneous or intramuscular injection, it stimulates the heart and produces a prolonged, sustained rise in the systemic blood-pressure.

My own practice has been to treat these cases as follows, in addition to laying the patient flat and giving aromatic spirits of ammonia diluted with water, as already mentioned: (*a*) The immediate intramuscular injection of 1 c.c. (15 minims) of adrenalin hydrochlorid (the usual 1:1000 solution) in the arm or gluteal muscles; (*b*) the immediate intramuscular injection of atropin sulphate in dose of $\frac{1}{50}$th grain. These are adult doses; children are given smaller amounts according to approximate weight. As a general rule, nothing more is required unless it is a second intramuscular injection of adrenalin in dose of 0.5 c.c. (8 minims) about ten minutes later. It is hardly worth while to give adrenalin by mouth. Whenever the physician has reason to expect the possible development of the acute vasoparetic reaction, adrenalin and atropin should be prepared beforehand ready for immediate injection and it is well for all physicians administering arsphenamin or its substitutes to have both drugs available and especially an *active* solution of adrenalin.

In severe cases accompanied by coma, it may be necessary to give adrenalin intravenously and for this purpose 0.1 c.c. (about 2 minims) well diluted with water or saline solution may be slowly injected; great care, however, is necessary when adrenalin is administered by this route.

In some cases persistent headache which may be accompanied by nausea, is very troublesome. These symptoms are probably due to increased intracranial pressure or "tension headache" for meningeal congestion and I have afforded prompt relief by the oral administration of sodium chlorid in dose of 1 gram (15½ grains) in salol coated pills or capsules every 5 or 10 minutes for 2 to 10 doses as suggested by Hughson[31] on the basis of the

investigations of Cushing and Foley.[32] Probably better results are to be obtained by the administration of magnesium sulphate as recently described by Fay[33] for the relief of intracranial pressure, in dose of 1½ ounces by mouth every fourth hour or 3 ounces in 6 ounces of water by rectal injection every fourth hour until the desired effect is obtained but with due care against producing excessive dehydration. In contrast with sodium chlorid, the magnesium salt is relatively non-diffusible through the intestinal wall, and, not being absorbed into the blood stream it exerts its entire effect on the vascular bed about the intestinal wall; hence, the direct extraction of fluid from the circulation and the secondary withdrawal of ventricular or tissue bound fluids bring about dehydration, for the circulation calls on all the available body fluids to compensate for the depletion in blood volume by way of the intestinal tract.

Treatment of Hemorrhagic Encephalitis.—These have been successfully treated by repeated spinal punctures (at least every twelve to eighteen hours) to reduce the pressure along with the intravenous and intramuscular injection of adrenalin. As soon as symptoms develop, spinal fluid should be drawn and 0.1 c.c. of adrenalin well diluted injected intravenously. It is well to follow this within ten to fifteen minutes by intramuscular injections of 0.5 to 1 c.c. of adrenalin and 1 c.c. of pituitrin. Urotropin in dose of 1 gram and caffein in dose of 0.2 gram in 15 c.c. of sterile water may be injected intravenously for dieuretic effects. Subsequent doses of these drugs must be given according to circumstances keeping in mind the physiological and toxic effects especially of adrenalin, and carefully avoiding over stimulation of the heart. It may be worth while to inject intravenously 1 gram of sodium thiosulphate or 3 grains of thiosinamin dissolved in 10 c.c. of sterile distilled water. I will shortly discuss the toxicity and action of these drugs with more detail in connection with arsenical dermatitis and jaundice. It would also appear that the intravenous injection of hypertonic solutions of sodium chlorid intravenously may reduce intracranial pressure, as suggested by the investigations of Weed and McKibben[34] and of Weed and Hughson;[35] the effect may be then prolonged by the oral administration of sodium chlorid or better still, by the oral or rectal administration of magnesium sulphate.

On the basis of the anoxemia hypothesis, it may be worth while to administer oxygen by continuous inhalation and the stomach may be washed out and sodium carbonate left in it on the assumption that acidosis may be partly responsible.

Gastro-intestinal Reaction. *Etiology and Symptoms.*—As previously stated, immediate nausea and vomiting sometimes follow the intravenous injection of arsphenamin, neoarsphenamin or sulpharsphenamin and especially if the injections are given soon after a meal; vomiting alone without nausea is not uncommon. Doubtless these effects are at times due to excitement and the ether-like taste and subside in a few minutes.

In a larger group of individuals, however, there are practically no immediate symptoms, but within twenty-four hours of injection and usually within one to four hours, gastro-intestinal symptoms develop among which nausea, vertigo, headache, thirst, vomiting and diarrhea are to be mentioned. Sometimes the temperature is elevated one or two degrees with pain in the extremities or "stab" pains in the back. These symptoms may be so mild as to merely make the patient feel "queer" or there may be repeated chills, numerous attacks of vomiting and profuse and protracted diarrhea.

I believe that reactions of this kind developing immediately or within a few hours of the time of injection are due to the vasoparetic effects of the

arsenic of the compound or of an impurity and that the symptom-complex is essentially a gastro-intestinal type of the acute vasoparetic reaction or "nitritoid crisis" in which the capillaries of the meninges and brain and of the intestinal mucosa are primarily involved. It is possible that a temporary acute cerebro-meningeal congestion is responsible for the headache, "queer" feeling in the head, vertigo, nausea and vomiting, the latter being due to a disturbance of the vomiting center; acute congestion of the gastro-intestinal tract with increased capillary permeability and serous exudation may be responsible for the "looseness of the bowels" or a well marked diarrhea, vomiting and thirst. There are no evidences of corrosive effects in the mucosa of the intestine and the symptoms are quite similar to those of acute poisoning from any arsenical compound.

Mild reactions of this kind are probably the commonest of all the toxic manifestations following the intravenous injection of arsphenamin and its substitutes. Women are especially likely to show symptoms of this kind and doubtless constipation and endocrine disturbances are important contributory causes. The incidence of reactions appear to be as high with neoarsphenamin as with arsphenamin but different lots of these compounds have varied greatly in the production of nausea, vomiting and diarrhea; that is, some lots of either produce more of these reactions than others, although no difference in toxicity for the rat could be determined. Not infrequently this gastro-intestinal reaction does not develop until six or more injections have been given suggesting either an accumulative toxic action of arsenic or the acquisition of hypersensitiveness to the drug. Sometimes the first course of twelve to fifteen injections of neoarsphenamin produce absolutely no ill effects whereas after the first injection of the second course following a "rest" of four to six weeks, nausea and vomiting may occur.

In addition to this type of diarrhea developing within a day or two of injection, there is another form which sometimes develops at a later time and in connection with dermatitis and jaundice. This type of diarrhea may be due to the effects of arsenic upon the intestinal mucosa, or as suggested by Stokes and Cathcart[36] to infection of the tract and especially of the colon with streptococci derived from areas of focal infection whose activities are stimulated by the surgical removal of these foci or by the administration of arsphenamin and its substitutes. I shall later refer again to this type of diarrhea in a discussion of arsenical dermatitis, as likewise to *stomatitis* which sometimes is present in association with the cutaneous lesions.

Treatment.—As previously stated in Chapter L, the gastro-intestinal reaction may be frequently rendered so mild as to prove free of annoyance if proper attention is given to the state of the bowels and particularly relief from constipation, proper hygiene of the mouth, and if the injections are given two hours or more after meals. When there is a tendency for the development of colic and diarrhea under these conditions I have found it advantageous to give 10 grains of sodium bicarbonate and 10 grains of bismuth subnitrate about an hour before injection repeating the bismuth if necessary upon the first signs of diarrhea. In severe headache, diarrhea and vomiting, it is sometimes necessary to administer adrenalin and I have observed prompt relief afforded by the intramuscular injection of 0.5 c.c. (8 minims) of the usual 1:1000 solution. In mild reactions of nausea, one or two vomitings and colic, relief is sometimes afforded by drinking an ounce or two of cold ginger ale or by sipping hot, well-seasoned broth.

In some cases it is necessary to give the patient a "rest" from arsenical treatment for several weeks or to reduce the dosage. Treatment can usually be continued with bismuth or mercury without ill effects and sometimes if

the reactions are being produced by arsphenamin, treatment can be continued with neoarsphenamin or sulpharsphenamin or the reverse.

Protein and Colloidal Shock Reaction. *Etiology and Symptoms.*—Frequently occurring with this gastro-intestinal reaction but sometimes developing alone from one to four hours after injection are a group of symptoms consisting of moderate fever (usually 100° to 102°F.), sometimes accompanied by perspiration, chilliness or a distinct rigor, headache and nausea which I believe may be due to a type of protein or colloidal shock reaction produced by the effects of intravascular hemolysis or the use of sterilized stale water or saline solution containing dead bacteria or molds and the products of their disintegration. During the early part of the reaction the leukocytes are frequently increased in number and since the reaction resembles that usually observed after the intravenous injection of typhoid vaccine, peptone and other protein substances in the non specific treatment of arthritis, etc., I have applied the term "protein shock reaction" to the syndrome since it appears to be a reaction apart from the effects of the arsenic of the compound.

This type of reaction is especially likely to follow the intravenous injection of solutions of disodium arsphenamin in water. While the plasma affords a considerable degree of protection against intravascular hemolysis, yet at the site of injection and particularly rapid injection with a large needle, it is likely that some hemolysis occurs as the solution comes in contact with the blood and sufficient for producing a mild reaction of this type. The reaction may occur likewise after the intravenous injection of neoarsphenamin and sularsphenamin and especially the rapid injection of the usual doses dissolved in 20 c.c. or more of water.

Hemolysis, however, is probably not solely responsible for these reactions. I am convinced, as stated in Chapter XLV, that reactions of this type may be caused by the use of stale distilled water or saline solution prepared with such water, sterilized before use of course, but containing dead bacteria or molds, the ill effects being due to the proteins of these organisms as stated by McIntosh and Fildes rather than to their toxins, which Wechselmann and other early writers on salvarsan reactions thought may be responsible ("Wasser-fehler"). Heating water and saline solution to 100°C. or higher is sure to destroy bacterial toxins, but is not sufficient for the removal of the proteins of the bacteria and molds themselves. The use of sterilized stale water (distilled or otherwise) is particularly important in this connection as such water may be contaminated with bacteria or molds and experience teaches that it requires but infinitely small amounts of these products injected intravenously to elicit shock reactions of this type. For this reason sterilized *fresh* water (preferably distilled) should be employed for the preparation of solutions for intravenous injection. Water freshly distilled sealed in ampules and sterilized cannot of course become contaminated as long as the ampules remain intact, but it is possible for stale water to produce reactions of this kind when the ampules are several months or years old and especially if chemical substances are dissolved out of the glass.

As previously stated, a colloidoclastic reaction of this kind is likely to occur along with reactions due to the arsenic and particularly the gastro-intestinal reaction; it is not always possible, therefore, to separate and recognize the protein or colloidal reaction as a distinct entity, but I believe that it is well to keep in mind the possibility of its occurrence alone or associated with reactions of other origin.

Treatment.—Mild reactions of this kind do not ordinarily require any special treatment and usually subside in an hour or two. Chilliness or a distinct rigor may require the patient to go to bed with the application of hot water bottles and to take hot drinks; a few drams of aromatic spirits of

ammonia in an ounce or two of hot water is sometimes helpful. In other words, the treatment is purely symptomatic and offers no difficulties.

Dermatitis and Allied Reactions.—Probably the most annoying of all the toxic manifestations following the administration of arsphenamin and its substitutes are the various rashes of the skin and mucous membranes with accompanying symptoms. The severe types of these reactions and especially exfoliating dermatitis, are likewise the most dangerous, as death not infrequently follows the development of these lesions. A very large literature has accumulated upon the subject since 1912 composed mostly of case reports and numerous classifications of the rashes, as well as theories of their etiology, have been proposed within recent years.

Classification of the Skin and Mucous Membrane Lesions.—As is now generally well known, quite a variety of rashes with subjective symptoms may follow the administration of arsphenamin, neoarsphenamin, silver arsphenamin, sulpharsphenamin and other organic and inorganic compounds of arsenic. Since the widespread use of arsphenamin and its substitutes in the treatment of syphilis the incidence of these reactions has naturally increased, but to the best of my knowledge none of the various skin rashes are peculiar to arsphenamin, neoarsphenamin or sulpharsphenamin alone and all have been described following the administration of various organic and inorganic compounds of arsenic before the discovery of arsphenamin and its substitutes.

The rashes that may follow the intravenous injection of arsphenamin and its substitutes are extremely varied and so many types may be encountered that it would appear almost useless to list them all on a morphological basis. As stated by Morrow[37] in discussing drug eruptions in general "a classification based upon the anatomical form of the lesions produced by drugs is impracticable. The very multiformity of the lesions forbids any such attempt, for while the irritant action of a particular drug may, in one individual be manifested by a single elementary lesion, the same drug may produce, in another individual, eruptive elements of dissimilar forms." The same is true of the skin lesions produced by the organic arsenicals now in common use for the treatment of syphilis. However, these reactions may be classified as follows:

- **Mild and early skin lesions**
  - Simple erythematomacular rash
  - Urticaria
  - Herpes
    - Simplex and especially of the lips
    - Zoster
  - Exacerbation of syphilitic rashes (Jarisch-Herxheimer reactions)
  - Pruritus
- **Sever and late skin lesions**
  - Dermatitis
    - Scarlatiniform erythema with desquamation
    - Erythema multiforme
      - Papular
      - Vesicular
    - Lichen planoid
    - Acute exfoliative
  - Purpura
    - Simplex
    - Rheumatica
    - Hemorrhagica
- **Mucous membrane lesions sometimes accompanying severe skin rashes**
  - Stomatitis and aplastic anemia
  - Exfoliative ileo-colitis
  - Bronchitis and broncho-pneumonia
  - Exfoliative conjunctivitis
  - Vaginitis
- **Chronic and recurrent skin lesions**
  - "Fixed" arsenical rashes
  - Pigmentation
    - Arsenical melanosis
    - Argyria
  - Arsenical hyperkeratoses
    - Palmar
    - Plantar

Stuhmer[38] has classified arsphenamin dermatitis under three headings: (1) Acute vasotoxic reactions, embracing those developing within a few hours to one or two days after injection as the acute, urticarial and erythematous rashes as well as the "fixed" arsphenamin eruptions; (2) subacute, anaphylactoid eruptions appearing from six to twelve days after the first injection and resembling serum sickness and also including the recurrent exanthems described by Nathan, in which the rash appears from ten to fourteen days after the first injection, fades within a few days and then partially recur; and (3) chronic dermatitis divided into early and late forms. The early form manifests itself after the fifth or sixth treatment; the late form, from several weeks to two or three months after the completion of a course of injections. These eruptions represent the severest types of dermatitis.

Incidence and Mortality of Dermatitis and Allied Reactions.—With so formidable an array of the skin and mucous membrane lesions known to follow the administration of the organic arsenical compounds one may readily enough acquire the impression that they must be of very frequent occurrence. But such is not the case insofar at least as arsphenamin and neoarsphenamin are concerned. As a matter of fact, however, the exact incidence of these toxic reactions cannot be expressed because the milder lesions do not ordinarily attract more than passing notice and with a few exceptions have not been reported. With the severer rashes, however, the incidence would appear to be not greater than one reaction to every 500 to 1000 injections of arsphenamin and neoarsphenamin as shown by the following reports; sulpharsphenamin however, has produced a higher incidence:

In 1917 Harrison[39] reported the occurrence of 124 cases of dermatitis in the course of 80,000 injections of arsphenamin to 10,000 patients. Of these twenty-six were severe, twenty four moderately severe, and seventy-four mild or fleeting. There were eight deaths or a mortality of 30 per cent. among the severe cases.

Parnell and Fildes[40] observed 38 rashes among 1250 men receiving 6588 injections of neoarsphenamin. Of these 12 were erythematous, 14 macular, 4 papular, 2 follicular, 1 polymorphic and 9 unclassified. In 2 cases, jaundice co-existed, but the rashes were mild in all but 1 case.

Moore and Keidel[41] have reported 23 reactions developing from 1914 to 1920 among approximately 9000 cases receiving about 47,000 injections of two different brands of arsphenamin, two brands of neoarsphenamin and one of sodium cacodylate. Of these 23 cases the rashes were mild in 5 and severe in 18, with 5 deaths or a mortality of 27.7 per cent. among the latter group. Moore in a personal experience covering 8000 injections of neoarsphenamin observed only nine cases of dermatitis, none being severe and with no deaths.

Stokes and Cathcart[42] have reported 38 cutaneous reactions of various types in the course of approximately 44,000 injections of arsphenamin in the Mayo Clinic. Of these 33 were studied in detail and 9 classified as severe dermatitis, 12 as moderate dermatitis and 12 as mild dermatitis. There were 2 deaths in the group of 9 severe cases or a mortality of approximately 22 per cent.

Sulpharsphenamin, however, may produce a higher incidence of dermatitis. Belding,[43] for example has recently reported an incidence as high as 16 per cent. in a group of adults and children treated with intravenous and intramuscular injections. My own experience with this compound has been similar, although the rashes have been mild with no cases of severe exfoliative dermatitis; the tendency of sulpharsphenamin to produce rashes is similar to that of arsenophenylglycin and for this reason I have learned to reserve the drug for the treatment of only those cases of syphilis to whom intravenous injections of arsphenamin and neoarsphenamin cannot be given. Of interest in this connection are the observations of Klauder[44] who noted a particular tendency for the development of dermatitis among patients in whom, through faulty venipuncture, some neoarsphenamin or arsphenamin was injected outside of the vein with resulting local reactions and suggesting that subcutaneous injections may be in some unaccountable manner particularly predispose to dermatitis. In my experience with sulpharsphenamin, however, the incidence has been as high after intravenous as after intramuscular injections and it can be stated that insofar as arsphenamin and neoarsphenamin are concerned the technic of administration bears no relation to the incidence of these reactions.

It would appear, therefore, that the general incidence of toxic skin and mucous membrane reactions after injections of arsphenamin, silver arsphenamin and neoarsphenamin is from 0.1 to 0.2 per cent. or one reaction to every 500 to 1000 injections. The mortality among the severe cases of the exfoliative dermatitis type has varied from 20 to 30 per cent. and for this reason exfoliative dermatitis is to be regarded as one of the most dangerous of the toxic reactions caused directly or indirectly by injections of the arsenical compounds.

Clinical Considerations of the Lesions and Symptoms.—Before entering upon a discussion of the etiology of these toxic reactions, it may be well to reverse the usual order of presenting the subject and first consider them briefly from the clinical standpoint which aids materially in an understanding of the causes.

As already mentioned in connection with the acute vasoparetic reaction or "nitritoid crisis" an *erythematous rash* is sometimes observed during or immediately after the injection over the chest and spreading to the neck and face. Sometimes this rash results in an urticaria but it usually disappears along with the other symptoms. More commonly a dusky red *erythematomacular rash*, blanching upon pressure, appears during the first week or two of treatment on the arms and legs and then extending over the body and sometimes involving the buccal mucosa. Slight transitory edema of the feet may accompany the rash as well as slight fever, malaise and nausea. As a general rule, the rash and symptoms disappear within two or three days with or without desquamation. It is stated by Moore and Keidel and others that further injections of the same or different drugs may be safely given, but I am personally always apprehensive of the development of one of the severer forms of dermatitis and never continue with the administration of the same drug but employ bismuth, mercury or another arsenical compound.

Urticaria may develop during the acute vasoparetic reaction or "nitritoid crisis" or within two or three hours after injection. In my experience it has usually developed after prolonged treatment with arsphenamin or neoarsphenamin, but may be seen after the first dose or two of these drugs and especially of sulpharsphenamin. The wheals last only a few days, but in exceptional cases may persist for a week. Aside from the itching, constitutional symptoms are not present. Having once occurred, urticaria is likely to occur again after repeated injections of the same drug, but in some cases it does not reappear; it is my practice, however, always to discontinue treatment with the drug responsible for the rash and employ another kind of arsenical compound which curiously enough may not produce the rash, or continue treatment for a while with bismuth or mercury before returning to the arsenicals.

Emery and Morin[45] and Saphier[46] have described cases of extensive urticaria accompanied by fever, adenitis and joint pains developing several days after injection and regarded as anaphylactic reactions because of the resemblance of these lesions to "serum sickness;" Moore and Keidel state that they have not observed reactions of this kind and neither have I up to the present time.

Herpes simplex and *herpes zoster* are rare types of rashes following the injection of arsphenamin and its substitutes. Herpes simplex may be severe, as in one case cited by Moore and Keidel in whom the chin, lips, tongue and buccal surfaces were literally covered with large herpetic lesions. Severe herpes, however, is rare although mild herpes is common but seldom noted since it causes little annoyance and the patient may not complain. Labial herpes is particularly likely to occur after the first dose, following which it

seldom reappears. Herpes zoster is rarer than herpes simplex and in some cases, at least, may be a coincident and accidental condition rather than a toxic arsenical reaction.

Herxheimer skin reactions in this connection refer to the accentuation of the rashes of acute early syphilis during the twenty-four hours following the administration of full doses of arsphenamin or one of its substitutes; with small doses the reaction may be avoided. The macular and papular syphilitic rashes become accentuated with increased reddening and swelling and occurring so soon after an injection may be mistaken for a toxic arsphenamin rash. As is well known, the same effects may be produced by mercury as originally described by Jarisch, Jadassohn, Herxheimer, Kraus and others.

Pruritus, or itching, is of particular importance as a prodromal symptom or danger sign of severe dermatitis if treatment with the arsenicals is continued. It is usually generalized and appears within twenty-four hours after injection, disappearing in a few days if treatment is stopped. Sometimes the itching is especially marked in the palms of the hands and may be accompanied by slight erythema. *Paresthesia* frequently accompanies pruritus and especially of the feet the patients stating that their feet feel "asleep;" the finger tips may be also involved and especially when the arms are stretched, but I will refer later to these symptoms in a fuller discussion of arsenical polyneuritis.

The essential lesion of the late and severe rashes is an inflammation of the skin—a dermatitis—developing one to fourteen days after injection with an average of 4.6 days as stated by Moore and Keidel in thirteen cases in which the time of appearance of the rash could be determined with certainty. As a general rule, the dermatitis develops during a course of treatment after six or more injections have been given; severe dermatitis has developed, however, after the first injection.

The rash may assume a wide variety of appearances responsible for the use of many different names for designating different lesions of essentially the same process. For example, the rash may appear suddenly as a *scarlatiniform erythema* of the extremities, gradually involving the trunk and disappearing in a few days, leaving a fine branny desquamation with entire exfoliation of the skin of the palms and soles of the feet; or the rash may be *morbilliform*, expecially in the trunk, or resemble the papular and vesicular varieties of *erythema multiforme*.

Sometimes the papules are entirely follicular, resembling *lichen ruber pilaris* and several writers, as McCaffery,[47] Frei and Tachan,[48] Buschke and Sklarz,[49] Smith[49a] and others have described cases in which the eruption closely resembled *lichen planus*. Sometimes the papular eruption is accompanied by a *pemphigoid eruption* of large vesicles and blebs which particularly involve the hands and feet.

These maculopapular and especially the vesicular eruptions, sometimes limited to the extensor surfaces of the arms and legs but more usually rapidly spreading until the whole body is involved, are followed by marked scaling and exfoliation—the condition well known as *dermatitis exfoliativa*. The skin of the entire body may be desquamated in large flakes and sometimes an entire palmar or plantar surface comes away intact. The rash is always accompanied by itching which may be intense and very distressing. Usually the exfoliation is complete in three or four weeks, but in some cases healing may take much longer and in occasional cases several exacerbations may occur in the same patient with exfoliation at intervals over several months. Fraser and Duncan have given the following excellent description of this severe dermatitis:

"The eruption usually starts on the flexor aspect of the forearms, next appearing on the face. In a very short time it spreads all over the body. At first it appears as a dark-colored scarlatiniform rash which rapidly becomes confluent. Small vesicles appear in the folds behind the ears, at the sides of the nose and on the neck. Blebs and pustules form all over the body, and their rupture causes crusting and scaling. Itching is very troublesome and is a source of infinite annoyance to the patient. There may be complete loss of hair on the scalp and shedding of the eyelashes and eyebrows.

"There is very extensive exudation into the skin and a characteristic puffiness and swelling of the face and head. The eyelids become so swollen and the face so puffy that the malar eminences practically meet the eyebrows, the eyes have disappeared from sight and the patient is unable to see. The conjunctivae are very infected and inflamed, the eyes feel sandy and lacrymation and photophobia are exceedingly troublesome. Transient blindness with edema of the optic discs may occur.

"A weeping eczema is usually present behind the ears, at the nasal folds and folds of the eyelids, and involving the lips, axillae, pubis, scrotum and perineum. Pyemic abscesses are common in severe cases and appear with alarming rapidity and persistence. Adenopathy is general and well-marked and occasionally the glands in the axillae break down.

"The skin at first becomes lobster-red and later reddish-brown. As exfoliation starts the skin settles down to a deep brown pigmentation and becomes thin and atrophic. The puffiness and general oozing and pain in the half-closed eyes and the very severe itching of the skin causes loss of sleep and the patient's health is soon seriously affected.

"In some cases constitutional symptoms may be slight but as a rule they are exceedingly severe. There is a high pyrexia accompanied by persistent headache, diarrhea, retching of the most trying severity and vomiting of bile-stained material. The tongue is dry, brown and heavily coated and the patient passes into the typhoid state. He is delirious and restless and often, if not closely watched, will get up and stagger about the room.

"In severe cases it is not unusual for the eruption to become hemorrhagic in places after the dermatitis has progressed for some time. When this happens the adenopathy is well marked and the glands particularly liable to break down.

"Lung complications are particularly fatal; pulmonary edema and bronchopneumonia are frequent complications in fatal cases.

"The patient becomes very emaciated and bed-sores are often trying and difficult to avoid or keep clean. Recovery is slow and convalescence protracted. When exfoliation is progressing the bed clothes are one mass of dry flakes and scales of considerable size. The skin thereafter is glazed, bronzed, thin and atrophic. Later it assumes a beautiful pinkish hue. The patient's appearance is so changed that his own friends may fail to recognize him. His features seem to have lost their proportion and he looks like an entirely different person. He returns to normal, however, in a month or two. The appetite returns sooner than one might expect and very often the patient will eat voraciously. Depression is a characteristic feature and often persists after the patient is eating well. Hyperkeratosis of the palms and soles frequently develops and these are the last to desquamate. Thick epidermal plates come away late during convalescence."

Polyneuritis and *jaundice* are sometimes present and *skin infection* with secondary rises of temperature are always likely to occur, especially in the weeping eczematous cases with cracks and fissures of the skin. It is sometimes impossible to prevent scratching and the introduction of staphylococci and streptococci which sometimes produce furuncles and carbuncles of serious import. Moore and Keidel, Stokes, and Cathcart and others have recorded several deaths from these secondary infections giving the severe types of this dermatitis a mortality of 20 to 30 per cent.

Of even more serious character is the development of *purpura;* two of four such cases recorded by Moore and Keidel succumbed. Jarecki[50] has classified these cases as those (*a*) showing small isolated hemorrhages from the gums, epistaxis and hemoptysis; (*b*) simple purpura; (*c*) purpura rheumatica and (*d*) acute purpura hemorrhagica which is very dangerous. Fortunately purpura is a rare reaction, but additional cases have been reported by Labbé and Langlois[51] Rabut and Owry[52] Florand,[53] Feit,[54] Calloman,[55] Weil and Wall,[56] Smith[56a] and others. More cases have occurred abroad than in America and neoarsphenamin has been apparently the most frequent cause; the one fatal case in my own practice occurred after eight injections of this compound. O'Leary and Conner[56b] however, have recently recorded two cases due to sulpharsphenamin.

In addition to these rashes, the majority of patients have an irregular and intermittent *fever* of 100° to 105°F. lasting for several weeks due to the secondary skin infections, focal infection, bronchopneumonia, etc.; also the accompanying increased pulse rate, respiratory changes, loss of appetite, etc. In relatively mild cases without focal or secondary infections the entire course of the dermatitis may be afebrile. Stokes and Cathcart have observed fever in a number of cases preceding the cutaneous eruptions and are of the opinion that this symptom is due in some cases to focal infection (apical abscesses, diseased tonsils, etc.) rather than to the cutaneous eruptions.

During the height of severe exfoliating dermatitis neighboring mucous membranes may share in the process as *exfoliating acute conjunctivitis, exfoliating vaginitis* and temporary *deafness* from exfoliation of the skin of the external auditory canal. *Diarrhea* due to acute ileocolitis occasionally develops during the course of the cutaneous reaction which may be due to arsenic or to secondary streptococcus infection as described by Stokes and Cathcart.

The exfoliating process may also involve the mucosa of the tongue and cheeks and severe *stomatitis* may complicate the cutaneous lesions. Stokes and Cathcart have reported 6 cases in their series; in 3 the stomatitis was mild and in 3 severe. One of their severest cases was accompanied by a relatively mild cutaneous reaction and in both cases reported by Moore and Keidel,[57] due to neoarsphenamin and associated with aplastic anemia, there was no dermatitis at all. In the mild cases there is little more than redness and dryness of the mucosa, but in the severe cases the gums, lips, cheeks and posterior wall of the pharynx may be involved with exudates, ulcers and hemorrhages due to secondary infection with the fusiform bacillis and spirochete of Vincent's angina.

Cases of stomatitis have also been reported by Görke[58] and Laurentier,[59] the latter drawing attention to the tendency for arsenical stomatitis to involve the fauces and pharynx with conjunctivitis and otitis while in mercurial stomatitis the front of the mouth and gums are especially involved. In dermatitis exfoliativa the *kidneys* are involved in about 80 per cent. of cases. Albumin and casts may be present in the urine and likewise blood in cases of purpura. Moore and Foley[60] have described four cases of severe reactions following the administration of arsphenamin with unusual blood pictures characterized by leukopenia, eosinophilia and an increase in the large lymphocyte and transitional groups, together with evidence of destruction of bone marrow. According to these investigators arsphenamin has a selective toxic and stimulating action on bone marrow and in one fatal case there was found at necropsy a markedly aplastic bone marrow showing degenerated cells and absence of the more mature forms of the myelocytic series. In the fatal cases studied by Moore and Keidel, necropsies showed markedly aplastic bone-marrow, with, as the outstanding feature, an almost complete absence of orderly leukopoietic centers. Nathan[61] has described leukopenia and a decrease of the polymorphononuclear neutrophilic leukocytes in cases of simple erythema and in severe dermatitis, leukocytosis due to increase of eosinophils and polymorphonuclears. Eosinophilia has also been observed by Hofmann,[62] Lathan[63] and by Stuart and Maynard[64] in cases of this kind and Feinberg[65] has reported an additional case of aplastic anemia following the administration of neoarsphenamin. Rosenbloom[66] has recently sounded a warning against the administration of arsphenamin in cases of hemophilia owing to the influence of this drug upon reducing the coagulation time of the blood. Desaux and his associates[67] state that a slight degree of acidosis may be present in severe cases of the arsenical erythrodermias.

I have reviewed these blood changes with considerable detail because I believe that they have an important bearing upon the etiology of arsenical dermatitis and the prognosis of individual cases since profound changes of these kinds in the bone-marrow may evidently reduce resistance to bacterial infection and readily enough introduce the factor of infection focal or otherwise, as one of considerable importance, as so well described by Stokes and Cathcart, to which reference will be later made in a discussion of the etiology of these reactions.

And now brief mention may be made of the more chronic and recurring lesions of the skin produced by the administration of arsphenamin and its substitutes. All of them have been known for many years before the discovery and use of arsphenamin in the treatment of syphilis following the administration of various organic and inorganic compounds of arsenic. Fortunately they are of rare occurrence and in view of the very large amounts of arsphenamin, neoarsphenamin and their substitutes administered to individual patients at the present time over comparatively brief periods it is a wonder that they are not more frequent.

Probably the most interesting of these are the so-called "*fixed arsenical exanthems*" described by Fuchs,[68] Schönfeld,[69] Leibkind,[70] Thibierge and Mercier,[71] Nagëli,[72] Kraus,[73] Guttmann,[74] Stokes and Cathcart,[75] Cabassi,[76] Klauder[77] and others. In some of these cases an urticarial patch on the arm, in the axilla, inguinal or popliteal spaces, on the chest or some other part of the body may develop after each injection followed by disappearance and the gradual production of a pigmented spot. As stated by Wise and Parkhurst,[78] these eruptions are very much like those seen in persons susceptible to antipyrin and phenolphthalein. They "consist of from one or more to several dozen sharply circumscribed, slightly elevated, smooth plaques, which may be pink, red, yellowish or brown; sometimes they leave no trace, at other times a moderate grade of pigmentation." The point of special interest in these "fixed" arsphenamin rashes lies in the fact that they tend to relapse and recur *in situ* after each injection. According to Stühmer they are caused by a combination of the "oxids" produced from arsphenamin in union with serum proteins which he calls "oxid-toxin."

In some cases the area becomes chronically inflammed with the production of a scaly, eczematous, lichenified patch. Eruptions resembling lichen planus following the injection of arsphenamin have been described by Kleeberg,[79] Buschke and Freymann,[80] Keller[81] and others.

Arsenical melanosis, or pigmentation, as well as palmar and plantar *hyperkeratoses* have been observed by Philip,[82] Hofmann,[83] Heller,[84] Klauder and others following the prolonged administration of arsphenamin and neoarsphenamin; in Klauder's case the pigmentation occured in the skin of the trunk of a man receiving a total of 100 injections of arsphenamin and neoarsphenamin before the discoloration became apparent. These lesions differ in no way from the lesions produced by other forms of arsenic which are of uncommon occurrence but well known and described.

Of special interest in this connection is the subject of *argyria* following the prolonged intravenous injection of silver arsphenamin and silver neoarsphenamin. As is well known the prolonged internal administration of silver nitrate and other inorganic salts of silver may result in local or generalized pigmentation or argyria; the organic silver compounds, however, are much less likely to produce this accident. Apparently when the silver exists in a compound in a non-ionizable form there is much less danger of the production of argyria and such is true in the case of silver arsphenamin and silver neoarsphenamin. Lochte[85] reported a supposed case of argyria following intravenous injections

of silver arsphenamin but apparently he did not see the case himself and it may have been one of arsenical pigmentation; Kogoj[86] more recently, however, appears to have observed a true case of argyria along with arsenical pigmentation in the case of a girl, age 23, receiving three courses of treatment of which the first consisted of 12 injections of silver salvarsan and the second of 11 injections of neosalvarsan and mercury. The same number of injections of neosalvarsan and mercury was given during the third course. This information was given by the patient herself and the exact quantities of the drugs injected were not ascertained. During the third course of treatment she noticed a gray discoloration of the whole body excepting the palms and soles, unassociated with any discomfort. When examined by Kogoj the mucous membranes of the mouth, lips, tongue and conjunctivae were of a diffuse, dark gray color; the genitals were of a dark brown color. Examination of sections of skin showed a marked increase of the melanotic pigment and the formation of dark brown granules; tests for arsenic proved negative. Kogoj thoroughly reviews the literature upon arsenical pigmentation and concluded that in this case the discoloration was due to arsenic as well as to silver.

Myers[87] has failed to produce argyria experimentally in rabbits and rats by a large number of injections of silver arsphenamin; in experiments conducted for me by Dr. Harkins consisting in the injection of rats with twelve or more doses of silver arsphenamin and keeping the animals exposed to sunlight, no discoloration of the skin or mucous membranes was apparent, although rats injected intraperitoneally regularly showed pigmentation of the parietal and visceral peritoneum.

In view of the fact that large amounts of silver arsphenamin have been administered in this country and abroad without the production of argyria, it appears that the danger of producing this distressing complication is very slight, probably on account of the chemical state of the silver in the molecule; but it cannot be said that the danger does not exist and I personally have always feared the possibility of this accident and have never given any single patient more than six injections. While silver arsphenamin may be slightly more spirocheticidal than arsphenamin the difference is slight at best and in my opinion it is hardly worth while to assume the chances of producing argyria with silver arsphenamin when equally good therapeutic results can be obtained with arsphenamin and indeed, with some brands of neoarsphenamin.

Kind and Dosage of Drugs in Relation to the Arsenical Dermatoses.—In the first place, it may be stated that all of the skin and mucous membrane lesions described above have been produced by various organic and inorganic compounds of arsenic and are not by any means toxic reactions peculiar to arsphenamin and its substitutes as neoarsphenamin and sulpharsphenamin; cases in illustration are those reported by Brooke and Roberts[88] from arsenical beer poisoning; of those reported by Putman[89] due to the arsenic in wall paper and a recent and striking case of severe exfoliative dermatitis following the administration of sodium cacodylate reported by Pusey.[90] It is not proper, therefore, to designate these reactions as "arsphenamin dermatitis;" rather they are *arsenical dermatoses.*

But unquestionably the various organic arsenicals vary somewhat in the productiveness of these dermatoses; arsphenamin is probably more likely to produce them than neoarsphenamin and sulpharsphenamin may produce more than either being somewhat similar to arsenophenylglycin in this respect. But the important practical point is that these lesions are produced by different arsenical compounds which naturally justifies the conclusion that it is the arsenic which constitutes the most important single factor in their

production. It is not possible, however, at present to work out a relationship between the chemistry of the compounds and the incidence of dermatitis and allied reactions, but as just stated, I believe that arsenic constitutes the toxic factor of most importance; it is practically certain that arsphenamin and its substitutes undergo profound changes after injection, but we do not know anything definite regarding the exact chemistry of these changes in relation to the production of skin lesions.

In a broad and general manner large doses of arsphenamin and its substitutes are more likely to produce these reactions than small doses, but there are striking exceptions in the case of individuals with natural hypersensitiveness to arsenic who react after the administration of small amounts. The same is true in the cases of those individuals who gradually acquire an intolerance during treatment, either in the form of acquired hypersensitiveness to arsenic or as a result of accumulation of arsenic in the tissues and especially those developing skin lesions, among whom the injection of small amounts may result in the production of dermatitis. Dosage, therefore, possesses only a very general relation to the production of these reactions and its influence may not be apparent at all, because it is not so much the amount of arsenic injected as the subsequent fate of the arsenic in the patient as modified by factors peculiar to individual cases.

Unquestionably, however, the arsenical dermatoses are more likely to develop among individuals who have received a number of injections than after the first dose although dermatitis has been reported after single injections. This suggests that in the majority of cases there is an accumulative toxic action probably a result of injury to the liver or kidneys or the acquisition of hypersensitiveness to arsenic, but in either case the fundamental changes occur fairly rapidly; in the series of 23 cases reported by Moore and Keidel for example, all but four occurred before the seventh injection and three occurred after the first.

Of further interest in this connection is whether or not the previous or simultaneous administration of mercury, bismuth and the iodids possess any relation to the production of arsenical dermatoses. Only 5 of the 23 cases reported by Moore and Keidel had taken mercury in conjunction with arsphenamin and they are of the opinion that the administration of mercury bears no relation to the etiology of the reactions; Stokes and Cathcart are of the same opinion. Wechselmann[91] and Eicke[92] and others abroad, however, are of the opinion that the administration of mercury may bear an etiological relationship by injury of the kidneys, resulting in deficient elimination; Schamberg[93] entertains the same belief. Ehrlich was also of the opinion that mercury, by damaging the kidneys, may interfere with the elimination of arsenic and thereby favor the production of toxic reactions and especially of hemorrhagic encephalitis. In my opinion, kidney involvement to the extent of interfering with the elimination of arsenic *may* favor the production of the arsenical dermatoses, although liver dysfunction is probably more important in this connection, but certainly the mere matter of administration of mercury is of little importance because both liver and kidneys may not be involved to the extent of interference with elimination. Strathy, Smith and Hannah[94] in reporting the 58 cases of delayed arsenical poisoning observed in an English war hospital, of which 8 had dermatitis, expressed the opinion that the simultaneous administration of gray oil was a predisposing factor and the histological studies of Lucke and myself, described in Chapter XLVI, on the changes produced by mercury in rats and rabbits, has lead me to the opinion that in some cases, at least, the administration of mercury may bear a relationship to the production of the arsenical dermatoses.

Syphilis and General Pathological States in Relation to the Arsenical Dermatoses.—Of considerable interest and importance in relation to this subject of etiology is whether or not the pathological changes produced in syphilis bear a relation to the production of the arsenical dermatoses. Clinically it is apparent that in the urticarias, erythemas and various papular, vesicular and exfoliative lesions that vascular changes in the skin are most prominent and it is well known that *Spirochæta pallida* brings about injuries of the endothelium of the capillaries which may intensify the vasculotoxic effects of the arsenic. Furthermore, it is possible that syphilis involving the liver and kidneys may interfere with the oxidation and elimination of arsphenamin and its substitutes and thereby throw added burdens of elimination upon the skin resulting in the production of lesions through increased vascular injuries.

The Jarisch-Herxheimer reactions of the skin sometimes observed during the treatment of cases of early syphilis in the secondary stage would appear to be due to a combination of the vascular injuries of syphilis plus the vasculotoxic effects of arsenic in addition to an increased affinity of the drug for the syphilitic exudates, and in these reactions at least there is a very definite etiological relationship between the vascular effects of syphilis and the vascular effects of arsenic.

However, the relationship of syphilis to the incidence of the arsenical dermatoses must not be over-emphasized. In the first place, the arsenical skin lesions may and have occurred in non-syphilitic individuals and the pathological changes of syphilis are not at all essential in their production. At best the most that may be stated is that diseased conditions in general including the pathological changes of chronic syphilis, and especially of the liver and kidneys, *may* favor the production of the arsenical dermatoses. In Moore and Keidel's group two of the severe reactions developed in 1800 cases of syphilis of less than one year duration or one in every 900 patients. Among 7200 late cases there were fifteen reactions or one to every 480 cases. These figures tend to show a higher incidence among cases of late syphilis which happens to correspond to my own experience, but Moore and Keidel state that if the probable statistical error is taken into account, considering the small number of cases of dermatitis, these figures do not show any special tendency for reactions of this group to appear either early or late in the disease. These investigators also observed that dermatitis was about three times more frequent in the white as in the colored race and state that since late vascular syphilis occurs with somewhat greater frequency in colored patients, there is no evidence to show that such vascular changes exert any influence upon the production of the dermatoses.

However, I cannot understand how we may escape from surmising that injuries of the liver and kidneys and especially of the former, by drugs or disease, to the extent of interfering with the metabolism and elimination of arsphenamin and its substitutes, may play a rôle in the etiology of the arsenical dermatoses and especially if it is believed, as I believe, that arsenic itself is the real primary etiological factor. Hoffmann[95] in 1911 maintained that the toxic reactions produced by arsphenamin were due to a perverted metabolism by a damaged liver and Glombitza[96] holds a similar view on the effects of syphilitic hepatitis. More recently Widal and his associates[97] have re-emphasized the importance of liver damage on the basis that a disturbance of the normal "proteopexic" functions of this organ by arsphenamin may be the basis of anaphylactic phenomena.

While it cannot be definitely stated, therefore, that injury to the kidneys, liver and other organs by drugs (arsphenamin, mercury and bismuth) or by disease amounting to an interference with the metabolism and elimination

of the organic arsenical compounds, bears a direct etiological relationship to the arsenical dermatoses, I believe that they *may* and if we regard the toxic skin reactions as due primarily to arsenic, dysfunction of these organs and especially of the liver, becomes a matter of considerable importance.

Bacterial Infection and Bacterial Anaphylaxis in Relation to the Arsenical Dermatoses.—Within recent years considerable attention has been given the possible relationship of bacterial infection to the arsenical dermatoses. It will be remembered in this connection that Wechselmann and others held that bacterial toxins in the water used for preparing solutions of arsphenamin may be responsible for some of the toxic reactions, although the dermatoses were not particularly mentioned in this connection. I do not think, however, that this theory commands much consideration at present although, as stated in Chapter XLVIII, the presence of dissolved bacterial proteins in stale water employed for intravenous injections may produce toxic reactions without any particular cutaneous manifestations.

Milian[98] has recently divided the dermatoses into two general groups on the basis of etiology believing that exfoliative dermatitis is the only skin reaction directly attributable to arsenic, while the others are due to the acute exanthemata as measles or rubella or the lighting up of latent bacterial infection following the temporary general lowering of resistance after the administration of arsphenamin. Moore and Keidel and Stokes and Cathcart, however, do not subscribe to the view that some of these skin lesions are simply coincident attacks of measles or other acute exanthems; for myself it be may stated that I have likewise never seen cases in which this hypothesis was substantiated, but Milian's view that latent bacterial infections may be stirred up by the administration of arsphenamin and its substitutes has been verified and is very important in relation to the etiology of these skin reactions.

Stokes,[99] for example, has studied the relationship of focal bacterial infection to these dermatoses, and especially exfoliative dermatitis, with great care and has shown in a clear and convincing manner that the presence of apical abscesses of the teeth, infected tonsils and other foci may predispose to the production of these reactions and more particularly, that an exacerbation of these or their surgical interference may precipitate a severe and even fatal dermatitis. Stokes and Cathcart have advanced the hypothesis on the basis of these clinical observations, that an allergic instability may result either from the continued absorption of a bacterial protein from a focus of infection or from the sudden liberation of such proteins following a flare-up in the focus due to arsphenamin as suggested by Milian. They also believe that it is possible for sudden sensitization to occur by an acute intercurrent infection. In addition to this state of bacterial allergy, they believe that the liver involved by syphilis or earlier injections of drugs may have its functions (proteopexic?) interfered with and thereby make the body unable to deal with the protein toxic substances from a focus of infection as well as with the arsenical metabolites of arsphenamin and its substitutes. They also suggest that arsphenamin may reduce the efficiency of the suprarenals and thereby produce a deficiency of adrenalin in the blood which destroys the vasomotor balance in the peripheral circulation and permitting a vasodilation. Their idea is, therefore, that patients may become anaphylactic to bacterial proteins from foci of infection; that injury to the liver may favor the production of this anaphylactic state and that injury of the adrenals by arsphenamin may result in vasodilation which permits bacterial proteins in the blood to reach the sensitized tissues and thereby produce anaphylactic shock expressed clinically by the various lesions of the skin. This idea was based upon the

observations of Auer,[11] who had previously shown that if the ears of rabbits sensitized to horse serum were rubbed with xylol to produce congestion at a time when the antigen was present in the blood, that an excessively severe reaction occurred suggesting that as a result of the vascular changes in the ear, an increased amount of antigen was brought to these tissues with the production of a local anaphylactic reaction.

When it is remembered that other forms of anaphylaxis and particularly anaphylaxis to foods among both children and adults is frequently associated clinically with various types of eczema, this hypothesis is deserving of very careful consideration. It is to be regretted, however, that Stokes and Cathcart overlooked conducting skin tests with the proteins of bacteria which could have been recovered in cultures of the focal infections in at least some of their cases; tests of this kind may have yielded additional information.

Moore and Keidel, influenced by these observations, offered an explanation of the mechanism of the arsenical dermatoses on the basis of a fundamental anaphylaxis to some foreign protein including the bacterial proteins as suggested by Stokes, but that the administration of arsphenamin or one of its substitutes produced dermatitis by the vasculotoxic effects of the arsenic which permits the access of protein antigen in the blood to reach the sensitized tissues and produce anaphylactic shocks; these anaphylactic reactions in turn produce greater vasodilation and thereby favor the deposit of arsenic in the skin and bone-marrow where it acts as an irritant. In other words Moore and Keidel believe that bacterial or some other form of anaphylaxis may be a fundamental change; that arsphenamin favors the production of bacterial anaphylactic shocks in the skin and that these in turn by producing vasodilation favor the deposit of arsenic in the skin which acts as an irritant. Both theories, therefore, regard bacterial infection as of fundamental importance in the production of the arsenical dermatoses and I believe that the theory advanced by Moore and Keidal is probably applicable to the majority of cases, since Fordyce, Rosen and Myers[101] and others have found an excess of arsenic in the scales and skin of cases of dermatitis.

Furthermore, the toxic effects of arsphenamin upon the bone-marrow with the blood changes described above undoubtedly tend to reduce resistance to bacterial infection and probably explains in part the occurrence of secondary streptococcus and staphylococcus infections in cases of severe dermatitis which may produce fatal terminations.

Arsenical Hypersensitiveness in Relation to the Dermatoses.—Hypersensitiveness to arsenic has been already discussed in relation to the general toxic manifestations of arsphenamin and its substitutes in Chapter XLVII; the subject is of particular interest, however, in relation to some of the dermatoses and for that reason is deserving of brief but special consideration.

It is well known that some human beings possess a natural hypersensitiveness to such drugs as quinin, cocain, procain and other medicinal substances and it is likely that similar state of natural hypersensitiveness to arsenic and mercury may be encountered. By natural hypersensitiveness I mean the condition of individuals displaying unusual or exaggerated susceptibility who have never before had the compounds administered to them. Whether or not human beings may acquire hypersensitiveness to arsenic by repeated doses, is not certain since the intolerance sometimes displayed after six or more injections of arsphenamin or neoarsphenamin may be due to cumulative toxic effects of arsenic.

But I am personally of the opinion that hypersensitiveness to arsenic may be acquired and especially of the skin. I happen to be well acquainted

with the case reported by Klauder[102] of a physician who developed asthmatic attacks and an erythematous scaly eruption of the fingers with occasional outbreaks of an eczematoid eruption on the face whenever he inhaled minute amounts of the powder of arsphenamin or neoarsphenamin during the opening of ampules or had some of the solutions come in contact with his fingers while preparing or injecting them. When the solutions were prepared by some one else and rubber gloves were worn during the administration, these lesions disappeared.

It is true that the hypersensitiveness in this case could not be passively transferred to guinea-pigs by injections of the patients' serum, but this is almost always the case in allergies to drugs; allergies to protein substances which I designate as true anaphylaxis are more successfully transferred to guinea-pigs by injections of serum, but allergies to non-protein substances as drugs, which I designate as hypersensitiveness, are practically never successfully transferred.

It is rather significant that Klauder's case had received an intracutaneous injection of 0.1 c.c. of either a 1:100 or 1:1000 solution of arsphenamin about eight months before the onset of the eczematous dermatitis and asthma which one naturally surmises may have been the sensitizing dose. As previously stated, Klauder has observed that individuals accidentally injected perivascularly with arsphenamin or neoarsphenamin during intravenous injections are especially prone to develop dermatoses and the incidence of the arsenical dermatoses is particularly high after the administration of sulpharsphenamin which is usually given by subcutaneous or intravenous injection. These observations naturally raise the question whether the skin is especially likely to become sensitized by subcutaneous injections of the organic arsenical compounds.

In Klauder's case the application of 2-per cent. solutions of arsphenamin and neoarsphenamin to abrasions of the skin resulted in positive reactions at the end of about four hours. Similar cutaneous as well as intracutaneous tests, however, conducted in 35 cases of syphilis under arsphenamin and neoarsphenamin treatment, a few of whom had recently recovered from dermatitis exfoliativa, yielded negative results. Stuart and Maynard[103] have tested 26 cases including three who had recovered from dermatitis exfoliativa, by intracutaneous injections of arsphenamin and neoarsphenamin in dilutions ranging from 1:1000 to 1:125,000 with positive reactions in two of the three cases of dermatitis. Moore and Keidel,[104] however, report negative cutaneous tests in cases of dermatitis; intracutaneous tests as advised by Stuart and Maynard were not employed.

These observations naturally suggest that in exceptional cases the arsenical dermatoses may be due to acquired hypersensitiveness to arsenic and whenever severe toxic reactions and especially dermatitis follows the first injection of small doses of either arsphenamin or neoarsphenamin properly administered and without evidences of kidney and liver dysfunction, one can hardly escape surmising that such reactions are due to natural hypersensitiveness to arsenic.

Author's Views on the Etiology of Dermatitis and Allied Reactions.—In my opinion the fundamental and most important etiological factor in the production of these skin and mucous membrane lesions is the effect of arsenic; I do not necessarily mean inorganic arsenic, but rather the effects of the intermediate organic compounds or metabolites or arsphenamin, neoarsphenamin, sulpharsphenamin, etc., although inorganic arsenic as contained in the arsenates and arsenites have been known to produce similar lesions. For example, the effects of arsenic upon the capillaries, described in Chapter

XLVI, are alone sufficient for explaining the simple erythemas and urticarias and its presence in the skin may explain the severer forms of dermatitis as well.

In exceptional instances the dermatoses may be due to natural hypersensitiveness to arsenic and thereby explain the rapid development of the lesions and symptoms after the first injection of a comparatively small dose of arsphenamin or one of its substitutes. Hypersensitiveness, however, is more frequently acquired and in such cases the administration of even small doses, after skin lesions have once appeared, may aggravate the lesions or precipitate a new attack. In some cases of mild dermatitis due to arsphenamin it is possible, however, to administer neoarsphenamin or the reverse without injurious effects; this suggests that hypersensitiveness has been acquired to a definite intermediate arsenical compound rather than to basic arsenic itself. But it would appear that in the majority of cases the skin lesions are due to cumulative toxic effects of arsenic, which may be foretold by various prodromal symptoms as excessive malaise, headache, diarrhea, erythema, etc., because skin tests do not ordinarily indicate the presence of hypersensitiveness and the lesions are aggravated or fresh attacks produced by the administration of any of the medicinal organic arsenical compounds.

Faulty metabolism of the arsenic or delayed elimination is probably responsible for the cumulative toxic effects and these may be due to injury of the most important organs concerned namely, the liver and kidneys, by the chronic pathological changes produced by *Spirochaeta pallida* in some cases, to bacterial or other diseases in others and to the administration of mercury in probably not a few. The skin is known to eliminate arsenic normally after the administration of arsphenamin and its substitutes and with deficient elimination by the liver and kidneys it is reasonable to suppose that more is brought to the skin for elimination where the excess acts as an irritant. In those cases of mild dermatitis due to arsphenamin in whom treatment may be continued with neoarsphenamin without ill effects, it may be that these cumulative toxic effects are not apparent because the latter compound is less toxic and more rapidly metabolized and eliminated than the former.

It is entirely reasonable to expect that the administration of arsphenamin and neoarsphenamin may stir up foci of latent bacterial infection just as they most certainly may stir up latent foci of *Spirochæta pallida;* under these conditions we may well expect in some cases to see the clinical effects of such increased bacterial activity as described by Stokes and especially if the arsenic has produced the bone-marrow and blood changes described by Moore and his colleagues, which still further reduces resistance to infection. Furthermore, there is no reason why we should not accept the hypothesis of Stokes that in some cases there is in addition to the toxic effects of arsenic in the skin the added effects of bacterial anaphylaxis characterized by additional vascular changes as is true in all forms of allergy in human beings, which may result in the still greater deposit of arsenic in the skin as believed by Moore and Keidel. Under these conditions the dermatoses and especially the severer kinds of dermatitis with exfoliation may be regarded as due to the irritant effects of arsenic in the skin alone or in combination with an acute bacterial anaphylaxis plus the elimination of toxic protein substances and a reduction in resistance or immunity to bacterial infection resulting in secondary local or general infections.

But I believe that it is arsenic which is the basis of the mischief. Arsenic is responsible for the primary toxic effects upon the capillaries and the resulting vasodilation and serous exudation shown clinically by various exanthe-

matous, macular and urticarial rashes. It may be likewise responsible for herpes, although in view of the recent investigations of Goodpasture and Teague[105] these lesions may be due to bacterial infection. It is the arsenic of the compounds which may induce a state of hypersensitiveness or accumulate in the various organs and tissues to be brought to the skin in either excessive amounts or in chemical state of enhanced irritating qualities. It is likewise probable that the arsenical substances of arsphenamin and its substitutes are the agents which may stir up latent bacterial infections and produce the toxic effects in the bone-marrow with resulting changes in the blood and reduction in resistance to secondary local or generalized bacterial infections. The mild dermatoses may be due, therefore, to the effects of arsenic alone; the severe dermatoses are due not only to the presence of arsenic in the skin, but in part to the effects of arsenic upon other organs and tissues and especially upon the liver, bone-marrow and foci of bacterial infection.

Prevention and Treatment of Dermatitis and Allied Reactions.—As a general rule, the arsenical skin reactions develop during a course of injections of arsphenamin, neoarsphenamin or one of its substitutes and various prodromal symptoms and signs may give warning of the approach of severe reactions if arsenic therapy is continued. Of these prodromata I have found itching, especially of the palms and soles and sometimes associated with numbness of the fingers, of most significance; some patients exhibit nervousness and irritability, extreme weakness and malaise, excessive diarrhea and headache. Mild and localized urticarial and erythematous rashes may appear; Moore and Keidel state that these do not preclude continuing with arsenic therapy, but I believe it is well to stop for a while all injections of arsenic upon the first appearance of any of these signs and symptoms and that this practice constitutes a very important phase of the prevention of the severe forms of dermatitis. Treatment may be continued with one of the compounds of mercury or bismuth without harm for one or two months and thereby permit the elimination of arsenic from the tissues. But in cases of severe dermatitis of the exfoliating type I believe it is well never to hazard further injections of arsenical compounds but to continue treatment with mercury, bismuth and the iodids. In the less severe forms of the dermatoses arsenical treatment may be resumed after this rest of one or two months, but always with a compound other than that producing the reaction, with small doses and with intervals of one to two weeks between injections until one is sure that ill effects are not being produced. For example, if arsphenamin has produced the reaction treatment may be continued with neoarsphenamin beginning with 0.05 to 0.1 gram.; if produced by sulpharsphenamin treatment may be resumed with arsphenamin or neoarsphenamin in dose of 0.05 to 0.1 gram. etc. But a very careful watch should be maintained for untoward effects and the injections stopped if itching, paresthesia, erythema, urticaria, etc. develop. Otherwise, the doses may be gradually raised by 0.1 gram. until the full dose is being administered. In addition it may be well to make total and differential leukocyte counts of the blood at frequent intervals as advised by Moore and Keidel; leukopemia associated with a reduction in the percentage of polymorphonuclear neutrophils with an increase of eosinophils and of the large mononuclear-transitional cells, or the appearance of many fragile or abnormal leukocytes, are to be considered of serious portent.

It might be worth while in cases of this sort first to employ intradermal tests for determining whether or not intolerance remains before resuming treatment with an arsenical. For this purpose I would suggest the *intra-*

cutaneous injection of 0.1 c.c. of 1:1000 dilutions of the drug which produced the untoward effects and the drug which it is proposed to use. If the reactions are negative forty-eight hours later arsenical treatment may be resumed but always with a different kind of arsenical than that which produced the dermatitis and always with small doses at longer intervals.

The treatment of gingivitis or surgical removal of foci of bacterial infection, and especially of apical abscesses of the teeth and tonsils, may be mentioned in connection with this subject of the prevention of arsenical dermatitis as emphasized by Stokes and his associates. But these precautions apply to the treatment of syphilis in general and will be referred to again in later chapters with more detail. It is well to emphasize here, however, that after signs and symptoms of dermatitis have once appeared such foci should not be disturbed until the danger of acute dermatitis has subsided, since Stokes has recorded instances of acute exacerbations with fatal generalized infections in instances of this kind.

Mild reactions offer no particular difficulties in treatment although the lesions and especially itching may be very annoying. Lassar's paste is excellent for application to herpetic lesions and for the relief of itching and burning of the skin and especially of urticaria, the frequent application of calamine lotion to which is added 5 grains of menthol and the same amount of phenol per ounce, usually affords relief. A brisk saline cathartic is also advisable as well as a simple diet of milk and vegetables. I also administer internally 10 grains of sodium thiosulphate (sodium hyposulphite) three times a day after meals, as this drug was originally advocated by Duhring for relief of urticaria and has since been especially advised for the treatment of arsenical dermatitis. Measures of this sort, along with prompt cessation of the injections of arsenic, are usually sufficient for the treatment of the milder dermatoses during the acute stages. Later, as the skin becomes dry and peals, it may be well to apply an ointment once or twice a day and especially to the soles of the feet where the fissure may be deep and liable to secondary infection; for this purpose one may employ any bland and soothing ointment to which are added 10 grains of phenol and an equal amount of calomel per ounce for the relief of itching and for bactericidal effects.

Severe dermatitis, however, of which dermatitis exfoliativa is the type, requires more vigorous treatment and careful nursing. The patient should be put promptly to bed and every precaution taken to prevent chills. A brisk saline cathartic should be given and daily evacuation of the bowels maintained in order to promote elimination by this route. The diet should be chiefly gruels, milk and milk preparations; meat and eggs should be forbidden. Plenty of water should be given in order to promote diuresis. The skin requires special care. A daily hot or warm bran bath or one containing a handful of washing soda may afford considerable relief from itching and especially when given during the evening hours. Calamin lotion containing phenol and menthol as mentioned above may be applied several times a day or if the skin is hot and dry, a soothing emollient is to be preferred as a mixture composed of 8 grams of gum tragacanth, 100 c.c. of olive oil and 140 c.c. of lime water. Moore and Keidel advise frequent changes of sterile night dresses and sterile sheets to lessen the dangers of infection. Sterile surgical stockings are also an aid and the hands may require bandaging to prevent scratching. McBride and Dennie state that daily exposure to sunlight is of great importance.

The mouth must be attended to with special care and likewise the face and eyes. Two or three drops of 25 per cent. argyrol should be dropped into each eye twice daily followed by boric acid solution. When the edema of the

face is excessive I have applied clothes wet with a saturated solution of magnesium sulphate with success and the same may be applied to other parts of the body with great relief.

Internally it is well to administer 1 gram of sodium thiosulphate (sodium hyposulphate) dissolved in water, three times a day; sodium bicarbonate may also be given internally or an alkaline drink of which the so-called Imperial drink is my favorite.

Ravaut[106] has stated that the oral and intravenous injection of sodium thiosulphate is particularly efficacious and the drug has been endorsed by McBride and Dennie[107] for the treatment of acute and chronic poisonings by arsenic, mercury and bismuth on the assumption that sulphur precipitates this group of metals and that sodium thiosulphate converts them into non-toxic insoluble sulphids and is especially serviceable because of its low toxicity. As stated in Chapter XLIV, Voegtlin and his colleagues have shown experimentally in rats that compounds containing sulphydryl groups (SH) as sodium thioglycollate and thiosalicylic acid reduce the toxicity for both rat and parasites (*Tr. equiperdum*) and they have advanced the theory that the toxicity of the trivalent oxids of arsphenamin, neoarsphenamin and their substitutes which are produced after injection of these drugs, is due to the fact that the arsenic of these are specific poisons for the SH group of glutathione and possibly other SH compounds which may occur in protoplasm. The logic of treating dermatitis with SH compounds would be to neutralize the toxicity of arsenic for the SH compounds of the protoplasm of cells and thereby to relieve the cells of injury; sodium thiosulphate, however, in their experiments, was found to be ineffective. McBride and Dennie maintain that this drug does afford relief and, as stated above, it is their belief that it acts by converting the arsenic in an insoluble sulphide.

One thing is certain and that is the very low toxicity of sodium thiosulphate. White rats can be safely injected intravenously with doses as high as 1 to 2 grams per kilogram of weight which are 50 to 100 times more than administered to human beings by McBride's and Dennie's plan of treatment which has been described as follows: "Given a typical case of arsphenamin dermatitis, the administration is as follows: 0.3 gm. of sodium thiosulphate is given intravenously; the following day, 0.45 gm.; the third day 0.6; the fourth day 0.9 gm.; the sixth day, 1.2 and the eight day, 1.8. This is usually sufficient for the severe case of arsenical dermatitis. Following the first dose, the redness, edema and serous exudate become lessened. The dry, desquamating stage is reached on the fourth to the tenth day. The entire course of the condition is shortened from three to six months to as many weeks."

Favorable results have also been reported by Sutton,[108] Miller,[109] Brigg and Fockoff,[109a] Harrell,[109b] Armuzzi,[109c] and others, but Greenbaum[109d] has observed much better results following the daily intravenous injection of solutions of *thiosinamin* in dose of 3 grains dissolved in 10 c.c. of sterile water. Thiosinamin in this dose is usually well borne but may produce a bitter taste and acid eructations; also digestive disturbances and lassitude fever.[109e] In animals Tyrode[109f] has described severe changes in metabolism with parenchymatous degenerations from relatively small doses, but in my experiments the repeated intravenous administration of the drug to rats and rabbits in dose of 3 to 10 grains (0.2 to 1.0 gm.) per 60 kilograms of weight produced no histological changes in the kidneys, liver or other organs.

Fraser and Duncan[110] have advocated daily intramuscular injections of *contramine* (*intramine*) in dose of 2.5 c.c. of a 1-per cent. emulsion. As many as twelve injections may be given; the local reactions are said to be but mild accompanied by a rise of temperature which subsides the following day.

The therapeutic effects may be enhanced by three or four intravenous injections of collosol iodin in dose of 50 to 100 c.c. French[111] and Rawlins[112] have also found this drug useful in many cases although it has occasionally failed to evert a fatal termination as reported by Fraser.[113] *Afenil* (chlorcalcium-carbamide) has also been advocated for treatment for subcutaneous or intramuscular injection in dose of 0.5 to 1 gram (5 to 10 c.c. of 10 per cent. solution).

Stomatitis if present may yield to treatment with sodium thiosulphate or thiosinamin as described above, but usually requires local measures as well and especially when severe. If smears show excessive numbers of spirochetes and fusiform bacilli it may be well to apply solutions of arsphenamin or neoarsphenamin locally several times a day. These do not appear to intensify the inflammatory reaction, but on the contrary may destroy the spirochetes with clinical improvement. For this local treatment I dissolve 0.2 gm. arsphenamin in 10 c.c. of boiling hot water and add 10 c.c. of glycerin. This mixture gives a 1 per cent. solution of the drug in 50 per cent. glycerin; alkalinization is not necessary. The solution oxidizes (darkens) upon standing, but this change does not reduce its local spirocheticidal activity. In addition it is serviceable to wash out the mouth several times a day with a 1:2000 solution of mercurophen, due care being exercised against swallowing the solution. The metallic tastes left by both arsphenamin and mercurophen can be removed with a final rinsing with cinnamon water.

In severe *aplastic anemia* treatment with sodium thiosulphate or thiosinamin should be given; transfusions with matched blood may be tried, but Moore and Keidel have not observed these to yield beneficial results.

Influence of Dermatitis on the Course of Syphilis and the Wassermann Reaction; "Esophylaxis."—In conclusion mention may be made of the observations of Hoffmann[114] who applied the term "esophylaxis" to a reversal of strongly positive Wassermann reactions to negatives, observed in certain patients after recovery from severe attacks of dermatitis, on the basis that the skin has a defense reaction which protects the internal organs. Buschke and Freymann[115] have also observed that the eczematoid eruptions with pronounced skin changes usually aids in the cure of syphilis with regression of lesions and a disappearance of positive Wassermann reactions. These observers are of the opinion that the skin may produce substances under these conditions destructive for *Spirocheta pallida*.

Bruck[116] has likewise subscribed to this view believing that an attack of dermatitis aids in some way the specific curative effects of arsphenamin and its substitutes just as *x*-ray and light therapy of gummata may aid specific medicinal treatment. Klaas[117] has also reported that in some cases recovering from severe dermatitis the Wassermann reaction is reversed to negative and may show no later symptoms and lesions of syphilis. Gougerot[118] cites several examples favoring this theory of "esophylaxis" and the effects upon the Wassermann reaction may be due to the tissues being more strongly impregnated with the drug than usual, that arsenic is retained in the body, or again that the fever which sometimes accompanies arsenical erythroderma has had a destructive effect on the spirochetes. Lesser,[119] however, disputes these claims and states that dermatitis does not permanently influence either the Wassermann reaction or the subsequent course of syphilis. Parounagian[120] reports a case in which the Wassermann reaction was still positive after the subsidence of severe dermatitis. Benveniste[121] states that the Wassermann reaction may be temporarily reversed to negative during the attack of dermatitis, but that this state lasts only a few months, is followed by positive reactions and that definite clinical symptoms and lesions of syphilis are likely to recur.

My own experience coincides with that of Benveniste; in some cases of severe dermatitis complement-fixation tests conducted according to my new method have yielded negative reactions, but in other cases these serological effects were not observed and in two cases of the first group the negative reactions were later followed by a series of positive reactions. All of these cases happened to be in the chronic stages of syphilis without external lesions so that I was not able to judge the effects of dermatitis upon the syphilitic processes. I believe, however, that it is entirely reasonable to expect some beneficial effects by reason of non-specific protein reactions either as a result of absorption from the skin or as a result of disturbances of protein metabolism by liver dysfunction. The fever and leukocytosis, especially in cases without bone-marrow changes, as well as the stimulation of enzyme and antibody activity, may cause regression of syphilitic lesions with consequent weakening or reversal of the complement-fixation reaction just as these effects in syphilitic cases may be produced by intramuscular injections of sterile milk and other protein agents or the inoculation of paretics with living malarial parasites to produce acute febrile reactions. Furthermore, as stated by Hoffmann[122] and Bloch,[123] in discussing the relation of the skin in general as a means of protection against the entrance of bacteria (exophylaxis) and recovery from infectious diseases, the skin may be a source of some internal secretion or the seat of production of enzymes and antibodies playing an important role in recovery from disease of the internal organs; in some cases of dermatitis it is reasonable to suppose that these functions may be enhanced, while in severe dermatitis they may be reduced with consequent increased chances of bacterial infection and graver prognosis.

Aside from these effects, it is, of course, possible that deficient elimination of arsenic may result in an enhancement of spirochetic destruction which would tend to bring about temporary clinical and serological improvement although there are no reasons for believing that the dermatoses are due to toxic substances from destroyed spirochetes.

At any rate it would appear that in some cases of dermatitis there may be at least temporary improvement in the syphilitic infection in compensation to the patient for a very disagreeable and even dangerous illness, but it is important for the physician to remember that these reactions do not result in spontaneous cures and that specific treatment should be continued with mercury or bismuth in conjunction with the iodids.

Neuritis, Neurorecurrences and Neural Herxheimer Reactions. *Paresthesia.*—During the administration of arsphenamin and its substitutes, some patients complain of numbness of the fingers and soles of the feet. In the hands the numbness is especially noticeable when the arms are stretched and the majority of individuals with these lesions state that the hands and feet feel "asleep." As stated in the preceding section, these symptoms frequently forecaste the development of dermatitis and sometimes accompany itching of the skin and especially of the hands and always indicate the necessity for stopping further injections of arsenical compounds until the symptoms have disappeared. After this has occurred treatment with the arsenicals may be resumed, but safely only with a different kind of compound than that producing the paresthesia, in small ascending doses at longer intervals between injections and with very careful surveillance of the patient.

It is my impression that these symptoms are produced by injections of sulpharsphenamin somewhat more frequently than by arsphenamin and neoarsphenamin. As a general rule, the symptoms develop after a number of injections of large doses have been given, but I have seen several cases develop

after single intramuscular injections of 0.4 gm. sulpharsphenamin and neoarsphenamin.

There is no pain and the reflexes do not appear to be involved. The lesions, however, are apparently those of a mild neuritis due to arsenic. As a general rule, the symptoms entirely disappear within three weeks after cessation of arsenical injections without permanent after effects.

Polyneuritis.—This neuritis is so mild that paresthesia may be the only symptom, but severe cases of polyneuritis are not unknown and especially after the administration of excessively large doses of neoarsphenamin, the symptoms and lesions being exactly similar to those produced by inorganic arsenical compounds. As early as 1912 Duhot[124] described five cases developing two to three weeks after the administration of four or five injections of neoarsphenamin in dose of 1.2 to 1.5 gm. each every two or three days. The patients first complained of pain in the soles and calves associated with dermatitis; later the planter, Achilles and patellar reflexes disappeared, associated with muscular weakness and even paralysis. Similar but less severe symptoms appeared in the hands and arms, but there were no disturbances of sight and hearing or of rectal or bladder functions. At about the same time, Wechselmann[125] stated that arsenical neuritis may follow injections of neoarsphenamin and since then additional cases have been described by Spillmann,[126] Sicard, Hagneman and Kudelski,[127] Solomin,[128] Pürkhauer and Mauss,[129] Bayet,[130] Schamberg and Greenbaum[130a] and others. Beeson[131] has also described a case of polyneuritis due to neoarsphenamin with flaccid paralysis beginning peripherally and extending centripetally, accompanied by muscular atrophy, prodromal pains in the extremities, marked pain in the affected parts on pressure, absence of bladder and rectal disturbances and Babinski's sign and with no pupillary changes.

Neoarsphenamin, therefore, appears to have produced more cases of polyneuritis than arsphenamin, but cases due to the latter have been reported by Variot and Bouquier[132] and by Abadie and his colleagues.

Toxic Amblyopia.—Unquestionably this neuritis is caused by the arsenic of these compounds. It is to be noted, however, that the optic and auditory nerves are not involved by the arsenic of the trivalent compounds, arsphenamin, and neoarsphenamin whereas the arsenic of the pentavalent compounds atoxyl and tryparsamid is especially likely to produce retrobulbar neuritis with transient dimness of vision or permanent amblyopias. Indeed, for this reason atoxyl has largely fallen into disuse as a therapeutic agent. Milian[133] has recorded three cases of visual disturbances following the intravenous administration of neoarsphenamin and believes that the myopia was due to a spasm of the ciliary muscles; he also states that whereas the arsenobenzols have an affinity for the sympathetic system, the oxygen compounds of arsenic (arsenic acid, arsenates) have an affinity for motor nerves, and phenylarsinic compounds (such as atoxyl) for the sensory, optic and auditory nerves.

Pearce[134] has observed cases of amblyopia treated with tryparsamid among patients with moderately or markedly advanced trypanosomiasis of the central nervous system, but not in the early stages of the disease; in a few cases there was permanent impairment of vision. Lorenz and his associates[135] observed dimness of vision in approximately 40 per cent. of their cases of neurosyphilis after four or five doses totalling 5 grams each. In all but two cases of paresis the amblyopia was transient. Moore and his colleagues[136] observed dimness of vision in fourteen patients among 150 cases treated with tryparsamid in doses of 1 to 3 grams at weekly intervals. In ten of these, the visual impairment was slight in degree and cleared up at once when the drug was stopped. In one, a patient whose original lesion

was syphilitic neuroretinitis, there was a slight permanent visual defect; while in three instances, disturbance of vision was severe. All of these fourteen cases had neurosyphilis, and in three there was present preexisting syphilitic disease of the optic nerve. They did not see any cases of amblyopia in early or late syphilis without involvement of the central nervous system, but ascribe the disturbances to arsenical retrobulbar neuritis and believe that, because of the extreme danger of causing amblyopia, the administration of tryparsamid ought to be confined to those classes of syphilitic patients in whom satisfactory therapeutic results cannot be accomplished with any other means.

Neurorecurrences and Neural Herxheimer Reactions.—While the various types of neuritis just described are due to the toxic effects of the arsenic of the medicinal trivalent and pentavalent organic arsenicals, other lesions of nerves and particularly of the cranial nerves may develop which bear an etiological relationship to the administraton of these compounds although they are not expressions of direct toxicity of arsenic on nerves; I refer to those lesions of the cranial nerves which develop after the cessation of treatment or at least late in the course of treatment. They are very probably due to the renewed activity of spirochetes escaping destruction, that is, in cases in which sterilization falls short of being complete. As explained by Erhlich[137] in 1911, the host fails to develop resistance against the spirochetes which survive in isolated foci and which are able to set up an intense lymphoid reaction; if this occurs in nerves or their sheaths symptoms are produced called neuro-recurrences. An analogous reaction has been observed by Bettmann[138] with cutaneous nests of spirochetes producing chancriform papules. Neuro-recurrences, therefore, do not indicate an actual stimulation of spirochetes or a direct action of arsphenamin on syphilitic processes. They occur weeks or months after the interruption of antisyphilitic treatment and are the result of resumed activity of temporarily suppressed spirochetes in patients who have been insufficiently treated and who have not been able to develop resistance. Occasionally a recurrent primary or secondary syphilis will develop at the same time.

Neurorecurrences, therefore, are principally observed in cases of early syphilis receiving insufficient treatment. Recently Gennerich[139] has described 16 cases, twelve of whom showed involvement of the facial or auditory nerves or a combination of these. Zimmermann[140] has recorded 23 cases with the following distribution, likewise showing this predilection for the facial and auditory nerves: optic, 3 cases; oculomotor, 1 case; trochlear, 1 case; facial, 3 cases; auditory, 5 cases; facial and auditory, 10 cases and 1 case each of oculomotor and facial, trigeminus and facial and trigeminus and oculomotor.

Pathological studies of neurorecurrences are not usually possible, but from cases examined by Pirila[141] and Lloyd[142] it would appear that the lesions may be either a diffuse meningovascular process or one limited to one or more focalized lesions.

According to Zimmermann the prophylaxis of such lesions depends on thorough mercurial treatment and it seems immaterial whether mercury is administered during or immediately following a course of injections of arsphenamin or neoarsphenamin. The lesions likewise tend to disappear under treatment, but it is necessary to give treatment with particular care, my practice being to start gradually with ascending doses of mercury or potassium tartrobismuthate before the administration of arsphenamin or one of its substitutes. The purpose of these precautions is to avoid the production of what may be called "neural Herxheimer reactions;" they were not

described by either Jarisch or Herxheimer, but the mechanism of their production appears to be the same as the original Jarisch-Herxheimer reactions which were the accentuation of the exanthems of the secondary stage. The nerves most frequently involved are the third, sixth and eighth cranial nerves. Reactions of this kind are especially likely to develop from the injection of large doses of arsphenamin (0.6 gm.) or neoarsphenamin (0.9 gm.) in cases of latent tertiary syphilis and especially of those with involvement of the oculomotor, auditory and other cranial nerves. Small ascending doses of these compounds are much less likely to produce these accidents as likewise mercury and bismuth; for these reasons the treatment of chronic syphilis should be begun gradually and cautiously rather than by the sudden intravenous injection of the maximum doses of arsphenamin or one of its substitutes. The reactions are not due to the effects of arsenic upon the nerves as is neuritis including amblyopia, but rather to the stimulation of syphilitic processes with sudden accentuation of lymphocytic exudates. Neurorecurrences develop after the cessation of treatment due to a resumption of spirochetic activity of parasites escaping destruction; neural Herxheimer reactions are due to a sudden accentuation of these processes by the administration of antisyphilitic drugs and especially arsenic.

Syphilitic and Arsenical Jaundice.—During the treatment of syphilis with arsphenamin and its substitutes, jaundice may occur and prove an annoying accident and the underlying lesions of the liver may even prove quite serious. It is natural for one at first to suspect the drug or drugs being administered as the cause of this untoward reaction, and especially since it is well established that arsenic administered in any form tends to accumulate in the liver and that it may produce degenerative lesions in the parenchyma of this organ. But it is to be carefully kept in mind that jaundice in syphilis may be due to the syphilitic infection itself or to the combined effects of syphilis and arsenic or indeed, may be entirely coincident and without any relation whatsoever to either syphilis or specific medication.

Classification of Jaundice in Syphilis and in Relation to Treatment.—The etiology of jaundice in syphilis may be, therefore, quite complex and in any given case requires very careful study and consideration from the standpoint of its treatment; the tendency is to jump to the conclusion that jaundice in the syphilitic under treatment is due to the drug or drugs being administered but this is not always true. Indeed I know of no other single symptom requiring more judicious study than jaundice in syphilis because if it is due to *Spirochæta pallida* it is obvious that logical treatment is by antiluetic drugs; however, if it is due to the drugs it is equally obvious that their administration should cease. On the ather hand, jaundice may be due to both syphilis and drugs, but the lesions may be very dissimilar and treatment therefore radically different according to the lesion: for example, it may be due to a combined hepatitis by *Spirochæta pallida* and arsenic in which case the administration of arsenic should cease; or it may be the result of the drug stimulating spirochetes into renewed activity producing what has been called "hepatic Herxheimer" and "hepato-recurrences," in which case antileutic treatment should be continued probably more vigorously than ever unless otherwise contraindicated.

And finally jaundice in the syphilitic may not be due to either the disease or the drugs being administered, but rather to accidental and coincident duodenitis, cholecystitis, cholelithiasis, etc., of other origin; in other words, the physician must exercise due care in guarding against the common error of ascribing all of the lesions and symptoms of disease in the syphilitic to *Spirochæta pallida* or the antiluetic drugs being administered; it is by no means

always easy, however, to differentiate these etiological factors and especially in late syphilis, but an etiological classification aids this important duty and the following has proven quite serviceable:

1. Due alone to syphilis
   - (*a*) In early syphilis (Icterus præcox lueticus)
     - Mild jaundice
       - Duodenitis
       - Hepatitis
       - Cholangitis
       - Pressure on common bile ducts
     - Severe jaundice—acute yellow atrophy
   - (*b*) In late or chronic syphilis
     - Chronic diffuse hepatitis
     - Chronic localized hepatitis (gummata)
     - Chronic perihepatitis
2. Due alone to arsenic
   - (*a*) By producing fatty degeneration, hepatitis and cholangitis
   - (*b*) By producing gastro-enteritis (obstructive jaundice)
3. Due to syphilis and arsenic
   - (*a*) Combined syphilitic and arsenical hepatitis
   - (*b*) Hepatic Jarisch-Herxheimer reactions
   - (*c*) Hepato-recurrence
4. Due neither to syphilis nor to arsenic
   - (*a*) Gastro-duodenitis of bacterial or other origin
   - (*b*) Coincident cholecystitis, cholelithiasis, etc.

Incidence and Etiology of Jaundice in Syphilis.—Jaundice occurring during the primary and secondary stages of syphilis is practically always due to infection with *Spirochæta pallida* and occurs in from 0.2 to about 2 per cent. of cases being somewhat more common in women, who appear to be in general more subject to other gall-tract injuries as cholelithiasis and cholecystitis which may be predisposing factors. As a general rule, the jaundice is of rather sudden onset coincident with the general eruption of syphilis and rapidly disappears under specific medication. This type of jaundice is usually mild and the underlying lesions may be quite diverse; for example, in some cases it appears to have been due to duodenitis, in others to local or diffuse syphilitic hepatitis and cholangitis and in others still to the pressure of enlarged lymph-glands upon the bile ducts. In other words, the mild jaundice of early syphilis is not necessarily an actual hepatitis or cholangitis, but I believe these are the usual lesions. Wile[143] has summarized the literature up to 1917 and it is hardly necessary to devote more space to the subject in this treatise, although I shall refer to it again in Chapter LXXV on the treatment of syphilis of the liver.

The majority of cases of jaundice in early syphilis are mild, but in approximately 10 per cent. of cases the jaundice may be severe and of the acute yellow atrophy type as reported by Gordon[143a] and others, in which the prognosis is very grave; this dreaded complication is, therefore, quite rare at the present time probably not occurring more frequently than once in 1000 to 2000 cases of acute early syphilis insofar as one may judge from the available literature upon the subject. Practically all investigators have agreed that in syphilis this is a true degenerative hepatitis and cholangitis due to *Spirochæta pallida* and its toxins. Acute yellow atrophy is, however, like the mild type of jaundice, more common in women, the proportion being about 5:1 and in some cases has occurred in pregnancy. Of course, one cannot be sure that predisposing factors are always present in these severe cases, but I strongly suspect that such is the case in addition to an unusually heavy infection of the liver with *Spirochæta pallida*.

It cannot be said, however, by any means that the incidence of jaundice in syphilis indicates the percentage of cases in which this organ is invaded and infected by *Spirochæta pallida*. Indeed, the incidence of hepatitic syphilis cannot be expressed at all because it is sure that infection may be present in the majority of cases without jaundice or other obvious symptoms. Neither can the incidence be expressed accurately on the basis of gross necropsy findings, because *Spirochæta pallida* may be present with the production of very small focal or diffuse lesions which cannot be detected with the naked eye. Only very care-

ful histological studies in the future will disclose the true incidence of hepatic syphilis, since Warthin's studies have shown the importance of microscopical examinations of tissues for evidences of syphilitic infection. McCrae[144] states that among 3300 autopsies at the Johns Hopkins Hospital there were 46 of well-marked syphilis of the liver, but in many of these there were no clinical manifestations. Rolleston quotes 64 cases among 11,629 autopsies at St. George's Hospital, London, and Flexner 88 cases among 5088 autopsies at the Philadelphia General Hospital. More recently, McNeil[145] found 64 cases of syphilis of the liver diagnosed during life in 1000 cases of syphilis; in 300 necropsies on syphilitic subjects involvement of the liver was found in 90. It is practically certain that the true incidence of syphilis of the liver is much higher; mild cases do not come to autopsy and most are sufficiently held in check by therapeutic measures to prevent chronic lesions of such character and size as to permit diagnosis during life. Based upon finding such gross changes in the liver as large gummata at necropsy, the incidence of hepatic syphilis in any large series of unselected subjects would appear to be from 0.5 to 2 per cent.; based upon combined macroscopical and microscopical studies as conducted by McNeil in unselected cases, the incidence is about 9 per cent. and combined examinations of this kind in necropsies upon syphilitic subjects has shown an incidence of 30 per cent. in the series reported upon by McNeil which comes closer to expressing the incidence of the infection of the liver with *Spirochæta pallida* in which evidences were discoverable at death.

Space does not permit a detailed description of the acute and chronic lesions of the liver produced by *Spirochæta pallida;* adequate descriptions are to be found in systematic works upon pathology and syphilis. It is, however, quite important for the physician to be acquainted with the signs and symptoms of acute and chronic syphilis of the liver and the gall-tract because the treatment of jaundice in syphilis demands this differentiation into cases due to *Spirochæta pallida* alone, to drugs alone, to a combination of *Spirochæta pallida* and drugs and lastly to extraneous and unrelated causes. In acute syphilitic hepatatis, jaundice may be only one of the signs; enlargement of the liver with tenderness, pain and belching after eating, etc., are usually additional important signs and symptoms. In chronic localized or diffuse syphilitic hepatitis, irregular or nodular enlargement of the liver, jaundice, epigastric pain and hepatic tenderness, ascites and enlargement of the spleen in some cases, symptoms grouped under the obsolete term "dyspepsia" etc., are usually additional signs and symptoms.

Incidence and Etiology of Jaundice Occurring in Cases of Syphilis in Relation to Treatment.—In any discussion of the incidence and etiology of jaundice in cases of syphilis under treatment with the organic arsenical or other medicinal compounds, it should be realized at the outset that in any given case the jaundice may be due to syphilis alone, to some other coincident and extraneous infection or pathological state alone, possibly but not at all probably to the drug alone, or most frequently of all, to various combinations of these circumstances.

Before considering these several conditions it may be stated, however, that the incidence of jaundice in cases of syphilis under treatment is very low; indeed, jaundice constitutes a relatively rare accident or complication although at some periods the incidence may be unusually high and above the average. For example, Stokes and Ruedemann[146] state that 70 cases of jaundice occurred in approximately 5200 cases of syphilis under observation in the Section of Dermatology and Syphilis of the Mayo Clinic from August 1916 to July 1920; this gives a case incidence of about 1.3 per cent. but 64 of these cases occurred between August, 1918, and July, 1920, suggesting to these investigators the possibility and probability that many were due to coincident infections apart from syphilis and the administration of arsphenamin. Bodin[147] has had a similar experience; of 254 cases of syphilis treated with neoarsphenamin between 1912 and 1914, only two or 0.78 per cent. developed jaundice; among 113 cases treated with a different brand during 1919 and 1920, it appeared in two or 1.77 per cent.; while in a third series of 472 cases treated in 1921, jaundice occurred in 34 or 7 per cent. All stages of syphilis were represented, and all soon recovered from the temporary jaundice. Holland[148] had likewise stated that the indidence of jaundice was greatly increased between 1920 and 1923.

In a statistical analysis by Meirowsky covering 225,780 injections of arsphenamin, neoarsphenamin and sodium arsphenamin, jaundice occurred once in each 2000 injections of arsphenamin (0.89 per cent. of cases) and once in every 6000 injections of neoarsphenamin. In 1915 Milian[149] reported 17 cases of jaundice among 800 cases of syphilis under treatment, an incidence of about 2.12 per cent. Five of these cases developed during treatment and twelve after the cessation of treatment constituting hepato-recurrences. Milian states that jaundice is particularly likely to occur during the second half of the first year of the disease.

Harrison[150] has reported the occurrence of jaundice in 0.6 per cent. of cases of syphilis during or subsequent to treatment with arsenical compounds. Morgan,[151] quoting Ravaut, states that in 94,762 injections of arsenical preparations in the treatment of syphilis in the French Army, in which the following preparations were used—novarsenobillon, 37,352, neosalvarsan, 35,836, salvarsan and arsenobillon 9,215, galyl 8,846 and luargol 3,523—there were twenty cases of jaundice which were divided among the various drugs as 7, 8, 1, 2, and 2 respectively. Fenwick, Sweet and Lowe[152] report two fatal cases out of 263 cases of syphilis treated by them. Scott and Pearson[153] state that among 2243 cases of syphilis treated with 13,664 injections of various arsenical preparations there were 39 cases of jaundice giving a case incidence of approximately 1.7 per cent.

Jaundice due to syphilis alone has already been discussed above and not a few observers as Tachan,[154] Lindstedt[155] French[156] MacCormac and Dodds[157] and others have expressed the opinion that jaundice developing in cases of syphilis under treatment is due rather to spirochetic infection of the liver with the production of widespread fatty degeneration and other changes than to the hepatic effects of the drugs.

But others are inclined to hold arsphenamin and its substitutes primarily responsible for the liver injuries. Golay,[158] for example, has reported three cases of jaundice produced by arsphenamin occurring in patients who were not suffering with syphilis and similar cases have been recorded by Pulvermacher,[159] Zimmern[160] and others. As a matter of fact it is frequently impossible to state in cases of syphilis whether the jaundice is due to the disease or some other incidental infection, to the drug or to a combination of these. The effects of antileutic drugs alone upon the livers of normal healthy human beings cannot be accurately determined, because the administration of these in repeated doses is practically confined to individuals with syphilis in whom the liver may be infected with *Spirocheta pallida;* accordingly we must resort to experiments on the lower animals and of these the white rat is best adapted because its diet can be made quite similar to the mixed diet of human beings.

Studies of this kind with arsphenamin and other organic compounds of arsenic have been described in Chapter XLVI. Undoubtedly large repeated doses may cause vascular and degenerative lesions of the parenchyma with some involvement of the bile ducts, but doses comparable to those given human beings on the basis of body weight produce none or but slight and inconspicuous histological changes. I believe it may be stated that the organic and inorganic arsenical compounds are capable of producing changes in the liver by reason of the storage and eliminative functions of this organ, but approximately normal and healthy livers of human beings are probably not injured in the great majority of cases by the usual forms of treatment with arsphenamin and its substitutes; intensive methods of administration are, of course, more likely to produce changes of these kinds than mild courses. But severe invasion and infection of the liver with *Spirochæta pallida* may so interfere with the metabolism and elimination of arsphenamin and its substitutes as to intensify their toxic effects upon the cells; I think the same may be true of other non-syphilitic infections and pathological states of the liver and under these conditions the production of hepatitis and jaundice may be facilitated and hastened. Jaundice, however, may be conceivably due primarily and entirely to arsphenamin and its substitutes by the production of duodenal changes leading to obstruction in the discharge of bile; as previously discussed, the arsenic of these compounds may produce vascular changes in the intestinal mucosa which in exceptional cases may result in the production of obstructive jaundice without involvement of the liver itself in the form of fatty degeneration, hepatitis and cholangitis. Of interest in this connection is the report of Oddo[161] who recently mentioned the frequency with which a history of syphilis is to be elicited in cases of catarrhal jaundice (in 11 out of 25 cases in his practice).

But doubtless, in the majority of instances jaundice occurring in syphilitic individuals under treatment is due to the summation of changes produced by the disease and the drugs if jaundice due to coincident bacterial infection (duodenitis) or other pathological states of the liver like cholelithiasis,

cholecystitis, etc., can be excluded. Syphilis of the liver undoubtedly predisposes to jaundice by interference with the physiological functions of the organ and especially the metabolism, storage and elimination of arsenic just as malarial infection of the liver predisposes to jaundice in cases treated with arsenic as stated by Heinrichsdorff.[162] Brocq[163] believes that arsphenamin and its substitutes may produce vascular and other injuries of the liver and thereby produce localized areas of reduced resistance favoring the localization of spirochetes which in turn may still further reduce the tolerance of the organ for arsenic and thereby set up a vicious cycle intimately involving the effects of infection and drug.

As stated in Chapter XLVI, it would appear that hepatic injuries following the administration of arsphenamin and its substitutes are caused by the arsenic of these compounds rather than by the benzene group of some non arsenical impurity. The liver is known to be an important storehouse of arsenic and in any case of fatal jaundice in which an excess of arsenic is not found in the bile or liver at necropsy one is justified is suspecting that the hepatitis has been caused by some kind of infection rather than by the drug administered.

It may be that the combined administration of mercury may aid in intensifying the hepatic effects of the arsenic of arsphenamin and its substitutes probably by a further interference with elimination by the production of nephritis, as indicated by the high incidence of jaundice (47 cases) in a series of 58 cases of delayed poisoning due to arsphenamin and mercury reported by Strathy, Smith and Hannah;[164] Lynch and Hoge[165] regard the concomitant administration of mercury as an important etiological factor in the fatal cases reported by them on the basis that faulty elimination intensifies the toxic action of arsenic on the liver. Foulerton,[166] commenting on his series of animal experiments, offers another explanation to the effect that mercury may exert a direct toxic action upon the liver already impaired by the toxic action of arsenic, but Wile, Stokes and Ruedemann, Pearson and Scott, previously referred to, have expressed the opinion that the administration of mercury has had no influence in their cases and my own more limited experience has been similar.

Jaundice, therefore, may be the result of a combined hepatitis and cholangitis due to *Spirochæta pallida* and arsenic. In some cases the syphilitic infection alone of the liver may produce no symptoms, but when arsphenamin or one of its substitutes is given, the lesions may be intensified constituting what has been termed as the "*hepatic Herxheimer reaction*" because the condition bears an analogy to the exacerbation of the skin lesions of the secondary stage of syphilis as a result of treatment as originally described by Herxheimer. Of the 39 cases of jaundice reported by Scott and Pearson, six were believed to be due to an effect of this kind in which they were of the opinion that the liver was probably swarming with spirochetes followed by the liberation of toxins following an injection of arsphenamin which produced degenerative lesions.

In other instances it may be that the administration of repeated doses of arsphenamin or neoarsphenamin provoke or stimulate latent spirochetic infection of the liver in a manner similar to the production of the provocative Wassermann reaction or as intercurrent infections, trauma, etc., may stimulate foci of latent spirochetes, and produce a form of jaundice due to the action of both parasites and drug, which is also sometimes spoken of as a "hepatic Herxheimer reaction." Cases of this kind, however, are more likely to fall into the group designated as "*hepato-recurrences*" or *hepato-recidives*" in which jaundice appears several weeks after the cessation of

treatment. In such cases it is believed that the drug has stimulated latent spirochetes into renewed activity with the production of an active syphilitic hepatitis and in a manner analogous to the production of active lesions of the optic nerve or other parts of the central nervous system when antileutic treatment is begun suddenly and vigorously in cases of chronic syphilis. Fabry and Wolff[167] and others have described cases of this kind and in the treatment of chronic latent syphilis of the liver, the administration of repeated large doses of arsphenamin or one of its substitutes may produce temporarily active lesions in this manner. Or in cases of mild syphilitic hepatitis without jaundice the functional capacity of the liver may be still further reduced by repeated injections of arsenic so that the cells cannot cope with the added toxicity of the arsenical compounds and severer lesions with jaundice result. Scott and Pearson reported 11 cases of this type in their series.

What appears to happen still more frequently is that the liver may be only very lightly infected with *Spirochæta pallida*, or may be free of this infection but have its functional capacity lowered by alcoholism or some other pathological state in which the added strain of repeated injections of arsenical compounds may result in the production of hepatitis and cholangitis with jaundice and the usual accompanying symptoms. Scott and Pearson classified 22 of the 39 cases in their series in this category. And finally, the fact that jaundice in syphilitic patients under treatment may not be due either to syphilis or the treatment is deserving of special consideration in view of the excellent investigations of Stokes and Ruedemann previously mentioned. From August, 1918 to July, 1920, the incidence in their experience suddenly increased over 1000 per cent. and their studies on these cases disclosed strong circumstantial evidence to the effect that an infectious agent, possibly associated with epidemic respiratory infections, was the exciting cause. Forty-one per cent. of their patients had coryza, tonsillitis, pharyngitis, influenza, etc., intimately associated with the prodromal symptoms or actual appearance of the jaundice and symptoms suggestive of systemic infection, with lassitude, arthritis and myalgia, backache, diarrhea or constipation, etc., usually preceded by several days or weeks the onset of the jaundice and other hepatic symptoms. Fifty-nine per cent. of their cases were associated with epidemics of colds and influenza and a total of as high as 73 per cent. yielded some contributory evidence of a possible infectious factor in the etiology of the jaundice. Stokes and Ruedemann have very properly stated the possibility of this type of jaundice masquerading as syphilitic or arsenical hepatitis with undue suspicion, perhaps, cast upon modern methods of treatment. This is especially true when no arsenic is to be found in the urine nor in the liver in necropsied cases. They admit that arsenic may be a predisposing factor, but that the exciting agent is as yet an unidentified infectious agent distributed by the blood and sometimes associated with gastro-intestinal lesions; Holland has likewise recently stated that in his opinion jaundice in cases of syphilis under treatment may be due to the fact that the arsenic produces gastro-duodenitis with ascending bacterial infection of the liver. Hallam[168] thinks that an infection resembling Weil's disease (spirochetosis icterohemorrhagica) should be considered as a possible etiological factor and the occurrence of jaundice among his syphilitic patients under treatment almost as an epidemic in 1917–1918 lends color to the suggestion, especially since it was proved that no particular batch of drug was responsible. This possibility is discussed also by Silbergleit and Fockler.[169]

Liver Functional Tests in Relation to Syphilitic and Arsenical Jaundice.—Since syphilitic hepatitis may not be suspected clinically until jaundice and its accompanying symptoms are produced, considerable interest is attached

to the possibility of developing reliable tests for the early detection of incipient dysfunction and jaundice. A reliable and clinically applicable test of this kind would prove of considerable value in the treatment of those cases of syphilis in whom hepatic involvement was suspected. In cases of marked syphilitic hepatitis, for example, preference in the selection of an antileutic drug would be limited to mercury and bismuth or, if one of the organic arsenical compounds were selected, extra caution would be indicated in dosage and intervals of injection in order to minimize or prevent the development of hepatitis and jaundice. From the standpoint of possible value in the diagnosis of syphilitic and arsenical hepatitis, these tests are, therefore, worthy of consideration in relation to the prevention of hepatic injuries and jaundice in relation to the treatment of syphilis.

Probably the best known of these methods is the *phenoltetrachlorphthalein test* originally developed by Rountree, Hurvitz and Bloomfield;[170] the return of the dye was determined by examination of the feces, but many discrepancies were noted by various investigators and since then the technic has been considerably modified. McNeil[171] has advised the collection of the dye through the duodenal tube and Aaron, Beck and Schneider,[172] Piersol and Bockus[173] Friedenwald and Gantt[174] and others have found this modification quite satisfactory. As shown by Wilhelm[174a] the duodenal lavage alone may be of value in the treatment of the jaundice.

Hoxie,[175] however, has found the results obtained by methods of this kind too irregular for reliance and more recently Rosenthal,[176] Rosenfield and Schneiders,[177] and Greenbaum and Brown[178] have advocated the withdrawal of blood at intervals after the injection of dye for the purpose of estimating the rate of its disappearance. This method consists in the removal of about 10 c.c. of blood for a control followed immediately by the intravenous injection of the dye in dose of approximately 0.005 gm. per kilogram of weight. Abel and Rountree[179] state that the dye in the dose recommended is non-toxic, yet Greenbaum and Brown observed occasional untoward effects in the nature of burning pain along the vein if injected too rapidly, late thrombosis, chills, faintness, sweats, and pain in the hepatic region. According to Rosenthal, a normally functioning liver will remove from the blood all but 3 to 7 per cent. of the dye in fifteen minutes after injection; in one hour, removal is complete. Recently Rosenthal[180] has shown experimentally in rabbits that the test can be detected following the removal of approximately 12 per cent. of the total liver substance of these animals.

Greenbaum and Brown found that in 70 to 75 per cent. of syphilitic individuals receiving from 1 to 124 intravenous injections of neoarsphenamin, that the results were normal. This indicates in a general way that neoarsphenamin as ordinarily administered does not produce sufficient disturbance of liver function to interfere with the elimination of this dye. Tests conducted with patients who developed jaundice showed that a more or less permanent degree of dysfunction followed clinical recovery.

Functional tests have also been based upon estimations of *urobilinogen* in the blood by Kahn,[181] for *hematoporphyrin* by Cavina,[182] the rate of elimination of *rose-bengal* (tetra-iodotetrabrom-fluorescene) by Delprat,[183] etc. But most attention has been given qualitative and quantitative tests for *bilirubin* in the blood.

This substance is a transformation product of hemoglobin and a great deal of discussion and an enormous literature have been devoted to the question whether or not it may be produced in tissues other than the liver. In 1886 Minkowski and Naunyn[184] reported a series of experiments with ducks and geese which were at once widely accepted as proof that there could be no formation of bilirubin from circulating hemoglobin without

the participation of the liver. In 1913 NcNee[185] reported essentially similar results observed with experiments on geese and stated that the pigment was a product of phagocytosis by the Kupffer cells of the liver and that it was not produced in animals from which the liver was extirpated except in very small amounts by similar cells in the spleen and bone marrow. Whipple and Hooper,[186] however, working independently of McNee but employing dogs, arrived at opposite conclusions and state that the liver is not necessary for the formation of the bile pigment from circulating hemoglobin. More recently, however, Rich[187] after repeating these and additional experiments arrived at the conclusion that the liver is necessary for the transformation of circulating hemoglobin into bilirubin.

The origin of bilirubin, therefore, still requires investigation but it is well established that small amounts are to be found in the blood of normal healthy human beings as 0.028 gm. or less per 1000 c.c. of blood and that it is increased in various diseases of the liver. Chargin and Orgel,[188] employing the qualitative method of Sunde[189] and the quantitative method of Meulengracht,[190] examined the blood serums and urines of 82 syphilitic cases under arsenical medication and observed that an increase of the bilirubin of the blood occurred in jaundice and served as a danger signal of its approach. Strauss and Buerkmann,[191] Wechselmann and Hohorst,[192] and Schneider[193] have also found that a disturbance of the liver by syphilis or drugs was accompanied or foreshadowed by bilirubinemia. Bernheim[194] has more recently described a method, for determining bilirubin in the blood which expresses the results in what is termed as the "icterus index," the technic being a modification of the Meulengracht method. Gerrard[194a] and Schamberg and Brown[195] have employed Van den Bergh's method[196] with success and this technic would appear to be widely employed. Bailey and MacKay[197] in a study of the blood and urine of 25 cases of jaundice following combined arsenical and mercurial treatment for syphilis in addition to 15 control cases, observed bile pigments in the urine of patients clinically jaundiced and showing bilirubinemia. An increase of urobilinogen was also noted and the oxidation of urobilinogen to urobilin was greatly reduced. Quantitative estimations of the blood for sugar, creatinin, uric acid and urea nitrogen yielded nothing of particular value although in the jaundiced cases there was an *increase of cholesterol* and Bailey and MacKay believe that its routine estimation may be of value in detecting the onset of liver injury in patients under treament since it is increased early and persists after other clinical signs have disappeared. Studies by Desaux and his colleagues[198] with the urine in cases of arsenical jaundice of the biliary acids, urobilin, urobilinogen, azoturic ratio, Arthus-Maillard's and Lanzenberg's coefficients of ureogenic imperfection, as well as of the residual nitrogen of the serum and its ratio to the total non-protein nitrogen, have indicated that hepatic disorders are due not to syphilis or the administration of arsphenamin but to such factors as alcohol, food deficiencies (especially of fats) or concomitant infections.

Finally mention may be made of the so-called *hemoclastic crisis test* devised by Widal, Abrami and Lancovesco[199] for determining the albumosestoring power of the liver as a test of its function. This is based upon the well known fact that after the ingestion of food there is usually an increase of leukocytes present in the blood. Widal and his colleagues observed that after milk has been given to patients with liver lesions, the usual leukocytosis does not occur, a decrease in the number of leukocytes usually being found. The phenomenon has been explained on the basis that the liver being unable to store up albumose the circulation is overcharged with the production of anaphylactic shock.

Normally in adults a mild degree of leukocytosis develops, but in liver dysfunction a leukopenia (digestion hemoclasis) may be observed in which the cells are 200 to several thousand less per cubic millimeter of blood than in the preliminary count. The test is not applicable to children. According to Widal and Abrami in almost 50 per cent. of their 432 cases, the crisis was over less than one hour after the ingestion of the milk. Einhorn[200] states, however, that in a number of cases of severe disease of the liver, the test has not helped to discover an insufficient action of this organ as in most cases there was a distinct digestive leukocytosis present, as in normal persons.

Practical Value of Liver Function Tests.—It is not yet possible to express an accurate opinion of the practical value of these various tests as aids in arriving at an estimate of the possible injury of the liver in syphilis or of other pathological states, by arsphenamin and its substitutes, etc. I believe, however, that they are worthy of serious consideration in cases of chronic active or latent syphilis in whom the presence of an icteric tinge of the mucous membranes or skin, gastro-intestinal symptoms, etc., lead to the suspicion of hepatic disorders and functional insufficiency. In cases of this kind evidence of dysfunction should lead to particular care in the choice of antileutic drugs and their administration and especially of the organic arsenicals, neoarsphenamin being less hepatotoxic than

arsphenamin. For the purpose of aiding in the diagnosis of such cases my preference is for the quantitative bilirubin test of the blood and the blood phenoltetrachlorphthalein test. Conducting the latter by duodenal drainage is quite a severe ordeal for most persons, although it is my preference when I believe something is to be gained in the way of bacteriological and cytological examinations of the bile by Lyon's methods as well as the possible therapeutic effects of the drainage. With slight modifications in the method of Piersol and Bockus described above it is possible to secure these additional data and effects.

Treatment of Syphilitic and Arsenical Jaundice.—It is obvious that the treatment to be given syphilitic persons with jaundice depends to some extent upon the cause of the conditions and an effort should be made to determine this as accurately as possible in each case.

The first question arising for consideration is whether or not specific medication should be started or continued, if already started; probably in the majority of cases of jaundice outside of those developing in the primary and secondary stages of syphilis, most physicians immediately stop the administration of antiluetic drugs but this may not be indicated in all cases.

Jaundice occurring in the primary and secondary stages of syphilis before any or but a small amount of treatment has been given, is usually so obviously due to spirochetic infection that one does not hesitate to administer arsphenamin or neoarsphenamin as a therapeutic measure; indeed, in severe jaundice of this kind (icterus gravis syphiliticus) the only hope for recovery is based upon intensive treatment with arsphenamin, bismuth or mercury as indicated by the recoveries of individual cases reported by Duhot,[201] Umber,[202] and others.

In those cases of *early* syphilis in whom jaundice appears after the first dose or two of arsphenamin or neoarsphenamin and regarded as hepatic Herxheimer reactions, specific treatment may continue, but if arsphenamin or neoarsphenamin is given it is well to reduce the dose and increase the intervals of injection or better still, administer potassium tartrobismuthate which is much less toxic for the liver and ranks next in spirocheticidal activity.

In those cases of late syphilis in whom jaundice appears several weeks after the cessation of treatment indicating the presence of a hepatorecurrence, it is well to interrupt specific medication for a month and then resume treatment, but in view of the fact that one cannot be sure that the hepatitis is not in part due to arsenic, it is my practice to administer ten or twelve doses of potassium tartrobismuthate by intramuscular injection in amounts of 0.1 or 0.2 gm. every four or five days along with small daily doses of potassium iodid by mouth. I think bismuth is to be preferred to mercury in cases of this kind because it is less irritating to the kidneys.

In the larger group of cases in which jaundice develops after five or more injections of arsphenamin or neoarsphenamin and in which we therefore suspect an arsenical hepatitis in addition to syphilitic infection, it is well to stop all specific medication for a month or six weeks at least. Milian and others are so confident that the jaundice is syphilitic, that they continue to administer arsphenamin or neoarsphenamin in many of these cases; but I must confess for my part a generous degree of temerity in following this practice. When I have reason to suspect that syphilis is very likely exerting an active rôle, I administer potassium tartrobismuthate by intramuscular injection in doses of 0.1 gm., but not usually until the patient has had a complete rest for at least one month. When treatment with the arsenicals is finally resumed, the doses should be small (0.1 to 0.2 gm.) and cautiously increased.

When jaundice is due to syphilis, it is clear, therefore, that active specific medication should be given and preferably by the administration of arsphenamin or neoarsphenamin for their prompt spirocheticidal effects. When a less active specific medication is desired and especially in cases to whom arsenical compounds have already been given, preference is to be given to bismuth or some form of mercury. In these cases in whom the jaundice is due to gastro-enteritis (obstructive jaundice) specific medication may cease altogether for at least a month. In those cases in whom the jaundice is believed to be due to arsenical hepatitis entirely or in large part, specific medication should be stopped and measures taken to afford relief from further damage by arsenic.

For this purpose, sodium thiosulphate may be administered orally and by intravenous injection in the same manner as described for the treatment of arsenical dermatitis. This compound is believed by Dennie and McBride to convert the arsenic into a sulphid, which is comparatively non-toxic and non-irritating. I have seen better results follow the intravenous injection of 3 grains of thiosinamin dissolved in 10 c.c. of sterile water every two or three days for several doses. Fraser and Duncan,[203] Scott and Pearson, French and others report good results following intramuscular injections of contramine (intramine-diortho-diamino-thio-benzene) in dose of 2.5 to 3 c.c. every three or four days for three to twelve doses as required. It is believed to act as a reducing agent and is said to act quickly. It usually produces some pain and stiffness and a slight rise of temperature sometimes follows.

Of course, if the jaundice is due to some intercurrent infection and especially to the presence of acute duodenitis, saline laxatives and other appropriate measures are indicated. Certainly it is not well to administer either sodium thiosulphate, thiosinamin or intramine unless there are reasons for believing that the jaundice is due solely or in part to the effects of arsenic.

General treatment applicable to practically all cases consists of the following: (*a*) rest in bed; (*b*) mild laxatives; (*c*) carbohydrate diet which increases the resistance of the liver; no fats and little protein, but milk may be given freely as likewise plenty of water and especially alkaline waters.

Accidental Perivascular Injections of Arsphenamin and Neoarsphenamin. Although intramuscular injections of arsphenamin and neoarsphenamin exert slightly greater therapeutic effects than intravenous injections, yet this route of administration produces so much tissue reaction, necrosis, pain and disability when full-sized doses are injected that it is only employed as present in exceptional cases, although the intramuscular injection of small amounts to children is feasible because the reactions are not excessive since the amounts injected are small.

Sometimes, however, solutions of arsphenamin and neoarsphenamin are accidentally injected into the tissues of the arm during attempts to administer them by intravenous injection. As a general rule, the occurrence of severe sharp pain and the production of a swelling about the site of puncture give warning, so that the injection may be stopped before a great deal has been injected; but when neoarsphenamin is being administered in a concentrated solution with a syringe, a few drops in the perivascular tissues suffices for the production of a very painful reaction. Sometimes the needle transfixes the vein so that a portion of the solution is injected intravenously and a small amount beneath the vein where the swelling cannot be as well seen and detected as in superficial injections. The severest reactions I have ever seen were due to the injection of very concentrated solutions of neoarsphenamin, as 0.9 gm. in 1 to 3 c.c. of water, into the perivascular tissues, the physicians believing the needles were in the veins and giving the injections so

rapidly that all were injected perivascularly before the mistakes were discovered by the development of excrutiating pains.

Symptoms and Tissue Changes.—If only a few drops of solutions of arsphenamin or neoarsphenamin have been injected perivascularly the patient complains of sharp pain followed by dull aching, which persists for several hours to several days depending upon the amount of drug injected. The tissues swell rapidly, the tumor possessing a peculiar hard or doughy consistency, rather sharply outlined with reddened skin. This swelling usually persists for many days and even weeks and months before it is gradually absorbed, the patient being partially disabled for most of this time as the result of pain upon flexion of the arm.

If larger amounts of drug are injected the pain is excrutiating and may radiate if the internal or external cutaneous nerves are involved. This sharp pain sometimes persists for several days along with a great deal of edematous swelling of the tissues including the skin. In a few days necrosis may occur with the ultimate breaking down of the tissues. I have seen cases in which portions of the capsule of the elbow joint were exposed by liquefaction necrosis of the tissues.

The chief tissue changes are muscle necrosis and leukocytic infiltration. The infiltrated muscle fibres become brassy, swell, loose striations and may ultimately liquefy. The capillaries are engorged with blood and interstitial hemorrhages may occur. As a general rule the leukocytes are unable to penetrate to the necrotic muscle but gather at the periphery which accounts for the rather sharp definition of the swellings. Absorption is delayed which probably explains the rather long duration of the tumefactions and their slow resolution. Ultimately fibrous tissue is produced, but without danger of ankylosis or disability unless a very severe necrosis has occurred when the possibility of partial ankylosis is not to be overlooked.

Treatment.—In the great majority of cases only a few drops of solution are injected perivascularly before the accident is discovered. The injection should be stopped immediately and the needle withdrawn. Sometimes a portion of the injected solution can be gently massaged out of the needle puncture of the skin. Wet compresses of a cold saturated solution of magnesium sulphate in water should be applied and the dressings kept moist for at least twenty-four to forty-eight hours or longer depending upon the individual circumstances. An ice bag may be laid over the dressings for the comfort derived and to retard swelling of the tissues. Sometimes hot solutions of magnesium sulphate give more relief than cold solutions and either may be employed.

Dennie recommends infiltration of the involved area with 2 c.c. of a 10-per cent. solution of sodium thiosulphate followed immediately by hot packs of a 5-per cent. solution of the same drug for several hours. If in spite of this treatment the severe infiltration still persists, he advises multiple stab punctures with applications of the hot solution. Dietel[204] has recently recommended injecting 10 c.c. of sterile saline solution into the site of the infiltration and surrounding tissues to dilute the arsphenamin, hasten its absorption and reduce irritation.

If large amounts of solution have been injected and especially a syringeful of a concentrated solution of neoarsphenamin, I believe it is well to incise the area as soon as possible under local or general anesthesia and gently massage out as much of the solution as possible. Due care must be exercised and especially of the internal and external cutaneous nerves. Hot solutions should now be applied either of 5-per cent. sodium thiosulphate in water or 1:3000 mercuric chlorid in hypertonic (10-per cent.) saline to reverse

osmosis as much as possible and to guard against bacterial infection and especially since the wound and sloughing tissues heal very slowly and are readily infected.

Reactions Produced by Intraspinal Injections.—In conclusion a few lines may be written of the cause and treatment of reactions following various methods of intraspinal therapy in neurosyphilis. In my experience the Swift-Ellis method, as described in Chapter LXII, is least likely to produce reactions, although some irritation of the lower cord with shooting pains in the legs may occur and render the patient very uncomfortable for a few hours at least and especially if an acute reaction of fever, chilliness, nausea and vomiting have followed the intravenous injection of the arsphenamin. Whenever I suspect that such a general reaction may occur, it is my custom to substitute an intravenous injection of 0.9 gm. of neoarsphenamin for the 0.6 gm. dose of arsphenamin, but otherwise proceed exactly as described. In my own method neoarsphenamin is now used exclusively in order to spare the patient the possible discomfort of a general reaction.

When arsphenamin or neoarsphenamin are added to serum or spinal fluid and injected intraspinally there is always more chances for lower cord irritation than when serum alone is injected as in the Swift-Ellis method. But by diluting the serum and drug with the patient's own spinal fluid and injecting very slowly by gravity, the degree of pain and discomfort can be greatly reduced and indeed, almost entirely prevented except for the soreness due to the puncture itself.

As a general rule, a feeling of discomfort with slight headache follows intraspinal injections; shooting pains in the legs are not infrequent due to the puncture or pressure, but these generally pass off rather quickly although in some cases morphin is required for relief. Fever and chilliness may develop; nausea and vomiting are not uncommon. Profuse perspiration may also occur, due more to nervousness than the drug, as is likewise the nausea.

By anesthesizing the skin, using a small bore needle (No. 19) and injecting slowly and cautiously, all of the discomfort and pain can be so greatly reduced that most patients do not hesitate or refuse a series of injections at intervals of two to three weeks.

In some instances, however, the ill or untoward effects are more serious and especially in tabes dorsalis. I have seen several cases of this type of neurosyphilis in which intraspinal treatments by the Swift-Ellis method have initiated gastric crises as well as loss of bladder and rectal control. It is my opinion that intraspinal therapy has definite risks and that this treatment should not be given except to selected cases in which thorough intravenous treatment with the arsenicals combined with intramuscular injections of bismuth and mercury by inunction have failed to arrest the progress of the disease. This phase of the subject is discussed, however, with more detail in Chapter LXXVI. The cause of untoward effects from intraspinal injections are probably due in part to disturbances in pressure and the choroid flexus, as well as direct irritation of the meninges and posterior roots of the nerves. In some instances serious respiratory and cardiac disturbances have developed during or immediately after injections and especially rapid injections with a syringe. Even sterile plain serum will irritate the meninges and produce congestion with serous and leukocytic exudations. Recently Kiely[205] has described a severe and fatal reaction due to the sodium citrate employed for the prevention of the coagulation of the blood in my method; I have never observed unusually severe reactions with this method probably due to the fact that I have been more fortunate in the sodium citrate employed but for over a year I have been using but one quarter the amount originally

advised with satisfactory results, the technic being described in Chapter LXII.

The treatment of reactions following intraspinal administrations is generally symptomatic. For those symptoms due to intravenous injections, treatment has already been described. For the shooting pains in the legs nothing much can be done except the hypodermic injection of morphin for severe pains. Of course the patient should be kept in bed for at least twenty-four hours; it is advisable to elevate the foot for an hour or two at least. An ice cap may be applied to the head if headache is complained of. In persistent headache large doses of sodium chlorid may be given internally for the purpose of reducing intraspinal pressure. If retention of urine occurs catherization is required until function is restored.

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CHAPTER LII

PHARMACOLOGY OF MERCURY IN RELATION TO METHODS OF ADMINISTRATION AND THE TREATMENT OF SYPHILIS

Although mercury is probably the most ancient of present-day medicaments for various ailments and diseases, and has been employed in the treatment of syphilis alone for more than five centuries, our exact knowledge of its pharmacology and especially the mechanism of its absorption and curative activity in this disease is quite incomplete. It may be said, therefore, that the use of mercury in the treatment of syphilis is still largely on an empiric basis. Within recent years however, the investigations of Wile and Elliott, Cole, Guttman and others in this country, and of Lomholt abroad, have contributed very materially to our exact knowledge of the amounts of mercury absorbed, retained and eliminated in relation to different routes of administration, but we are unable to corelate the results with animal tests

for curative activity which have proven so helpful in elucidating the subjects of the mechanism of absorption and curative activity of the organic arsenical compounds, because mercurial compounds are almost without demonstrable trypanocidal activity even in maximum tolerated doses and of relatively feeble spirocheticidal activity in experimental infections.

Absorption and Elimination of Mercury Administered by Ingestion. Absorption from the alimentary canal may be first considered, especially since the administration of such soluble compounds as mercuric chlorid and the yellow iodid (protiodid of mercury) are so commonly employed during the treatment of some cases of syphilis; furthermore, when mercury is administered by the methods of fumigation, inhalation and inunction, a portion is probably swallowed in the saliva after condensation of the vapors in the mouth and upper respiratory tract. Moreover, since mercury is eliminated in the bile and possibly by excretion by the glands of the intestinal mucosa after administration by all methods, it is entirely likely that a portion is re-absorbed before passage in the feces, accounting in part, at least, for the very long periods of elimination commonly observed after the administration of mercurial compounds. As stated by Fournier, the administration of mercuric chlorid and the protiodid constitutes the most practical method of administration when mercury is to be given over long periods of time.

A large number of mercurial compounds have been advocated for oral administration and the older literature is full of numerous potions and special formulas. In the first place, it may be stated that preference is always given the soluble preparations. Metallic mercury is absorbed so slowly that it is apt to be passed before appreciable amounts have become oxidized and converted into soluble compounds or before sufficient amounts may be volatilized and directly absorbed through the intestinal mucosa. Calomel, however, may be quite readily absorbed presumably after being broken down by the alkaline intestinal juices into metallic mercury and yellow mercuric oxid, the latter slowly dissolving in the intestinal contents; whether or not mercuric chlorid is produced under these conditions is still undecided. In this connection it may be stated that Beinhauer[1] found, by analysis of the urine of patients according to the method of Elliott,[2] that excretion of mercury began from six to twelve hours after the ingestion of ¼ to 2 grains of calomel in doses of ¼ grain every 15 minutes. The rate of excretion was the same for large and small doses and the investigation has proved of interest as showing how rapidly this compound may be absorbed. Lomholt[3] states that a rapid saturation of the body by mercury may be secured by the oral administration of 0.5 gm. (about 7 grains) of calomel; Guttman has found that metallic mercury triturated into small globules in food and fed to guinea-pigs results in absorption and the production of toxic tissue changes, whereas, when given in mass, there may be little or no absorption and no toxic effects.

As compared with administration by inunction, inhalation and injection, absorption from the alimentary canal is much less active; Lomholt states that in a few examinations of the urine made by him the degree of absorption, as judged by the degree of elimination, was very small; details of his experiments were not published. Clinical experience has shown quite conclusively, however, that when mercuric chlorid, the protiodid and other soluble compounds are administered orally in the doses advised by Fournier, considerable amounts are absorbed as indicated not only by such toxic effects as stomatitis and renal irritation, but likewise by curative improvement of syphilitic lesions. Of course, the production of local irritive effects, as "sublimate gastralgia," colic and diarrhea, is not acceptable as evidence of absorption, and the catharsis may indeed materially interfere with absorption by increas-

ing peristalsis and exudation from the intestinal mucosa unless controlled by opium. Nothing is definitely known of the rate of absorbability of different compounds of mercury from the gastro-intestinal canal. Apparently, solubility bears no important relation to the process as shown by the rapid absorption of calomel and the protiodid which are insoluble in water and other vehicles. Off hand, one would expect that soluble preparations, as the bichlorid, would be most rapidly absorbed, and this is probably the case; but this compound is likely to be converted into the insoluble albuminates and sulphids when administered orally, although its rapid absorption from the vagina indicates that it may be absorbed directly and with great rapidity. Fistula experiments have shown that absorption of all compounds occurs principally in the ileum, but is also known to occur in the rectum and doubtless may take place, to some extent, anywhere along the entire gastro-intestinal canal.

Mechanism of Absorption of Mercury Administered by Ingestion.—As previously stated, metallic mercury is not commonly administered as such in pills for the treatment of syphilis, but nevertheless some is swallowed by patients when the inunction and fumigation methods of administration are employed. As will be shortly discussed, it would appear from clinical experiments as well proven that a portion of the mercury applied to the skin in the form of an ointment of the metal (*unguentum hydrargyri* or of calomel) is volatilized by the heat of the body and inhaled, following which a portion is doubtless swallowed in the saliva; the same is true to some extent after fumigation. What is the subsequent fate of this metallic mercury? Are soluble compounds produced, like mercuric chlorid? Numerous authors state that this takes place, but the evidence is not convincing. Even if soluble compounds are formed, may not insoluble albuminates and sulphids be produced in the intestinal contents and mucosa, as is believed to occur after the administration of such soluble salts as mercuric chlorid and the biniodid?

It cannot be said, therefore, that the mechanism of absorption of mercury from the gastro-intestinal canal has been satisfactorily explained. It is likely that by oxidation and other chemical reactions soluble compounds are gradually produced from metallic mercury, albuminates and sulphids, etc., and that these are directly absorbed with the products of digestion into lymphatic and venous channels. But in addition, I believe that a part is directly absorbed in colloidal suspension in the form of minutely divided particles after volatilization and distributed as such by the blood or after oxidation and conversion into true solutions in the blood and other body fluids.

Absorption and Elimination of Mercury Administered by Cutaneous Application.—As stated by Fournier, the administration of mercury by contact with the skin is one of the oldest of all methods of administration. Inunction was employed in the fifteenth century and is mentioned by Fracastor in his poem on syphilis. But, administered in a reckless fashion, "anointed to the point at which the teeth started like the keys of an organ when played on," the method soon led to a violent reaction against it.

Mercury may be administered by this route not only by rubbing ointments of calomel and other insoluble compounds into the skin, but also by the method of fumigation, by wearing mercurial plasters and impregnated flannels or taking tepid bichlorid baths (method of mercurial balneation). Only the inunction method is now commonly employed. Absorption of both soluble and insoluble compounds of mercury may likewise occur from wounds and after applications of solutions and ointments to syphilitic ulcers.

Absorption and Elimination of Mercury Administered by Inunction.—For the inunction treatment of syphilis a drahm of the official blue ointment (*unguentum hydrargyri*) is usually applied; this dose contains 30 grains or 50 per cent. metallic mercury in a finely divided state. Schamberg has employed an ointment containing 45 grains of calomel in a dose, as equivalent to this amount in terms of metallic mercury since calomel contains 85 per cent. of the metal. At the present time both ointments are employed, but the blue ointment to a greater extent than the latter since metallic mercury is more volatile than calomel, is therefore absorbed more readily after cutaneous application and produces better therapeutic results, as will be discussed shortly with more detail.

Before entering upon a discussion of *how* mercury is absorbed when applied to the skin—that is, whether it is absorbed directly or after volatilization and inhalation or by a combination of these two routes—I shall briefly summarize recent experimental studies on the elimination of mercury applied by inunction as an index of the degree of absorption. Wile and Elliott,[4] in a study of the rate of absorption of different salts of mercury, found, after inunctions with 4 gm. of 50 per cent. ointment of mercuric salicylate, 3 gm. of 50 per cent. ointments of the yellow and red oxids and 4 gm. of a 50 per cent. ointment of the oleate, that mercury could be found in the urine in from 48 to 72 hours later, but never in as large amounts as follows inunction with the official ointment of mercury (*unguentum hydrargyri*). Of course a part of the difference in elimination of mercury in these experiments may have been due to variations in the dosage calculated in terms of metallic mercury. But aside from this consideration, additional experiments showed that these compounds have a lower vapor pressure, that is, are less rapidly volatilized at body temperature, than metallic mercury, calomel and ammoniated mercury and that, while inunction is followed by absorption probably directly through the skin, inunction with ointments of compounds more readily vaporized is followed by a greater degree of absorption due to the inhalation and absorption of mercury vapors in addition to that absorbed directly through the skin. In a subsequent study of mercury following inunctions with blue ointment and calomel of presumably equal amounts on the basis of mercury content, Wile and Elliott[5] found mercury in the blood only after a considerable number of inunctions of blue ointment had been given; after a similar number of inunctions with calomel ointment mercury was not found in the blood, indicating that the mercury of blue ointment is absorbed into the blood more rapidly than the mercury of calomel. Inunction with both ointments resulted, however, in the presence of mercury in the milk of nursing women, in saliva and stomach contents, but not in the cerebrospinal fluid. In all instances, however, absorption of mercury from calomel ointment appeared to be slower than from blue ointment, as determined by the rate of elimination.

Lomholt studied the elimination of mercury in the urine and feces in two patients receiving daily inunctions of blue ointment in dose of 3 gm. In both cases a slow but regular increase of the elimination was observed during the first three weeks; afterwards the elimination kept almost stationery at bout 0.003 gm. per day. Even forty days after stopping the inunctions, elimination exceeded 0.0005 gm. per day. The average elimination per day under treatment was 0.00217 and 0.00215 gm. of mercury respectively; the quantity eliminated in the feces amounted to 26.2 and 30.8 per cent. of the total respectively for these two cases, but elimination through the feces was very irregular and without any remarkable increase under treatment.

These and additional studies by Ramsey and Ziegler,[6] Schamberg, Raiziss, Gavron and myself[7] and others have shown, therefore, that application of mercury to the skin is followed by rapid absorption as shown: (1) by the appearance of mercury in the urine and feces; (2) by the production of toxic effects, especially stomatitis and (3) by the production of therapeutic effects which are frequently well marked. The clinical effects alone are so well known that investigation on the subject of elimination was never really required to show that absorption occurred but rather to indicate which preparations of mercury are best absorbed and especially the mechanism of absorption.

Absorption of Mercury Administered by Fumigation.—The treatment of syphilis by mercurial fumigation is nearly as old as the inunction method.

It was in great favor in olden times, and, known as the "treatment by perfumes," constituted an means for securing the "good salivations" which were then considered necessary for eliminating the "corrupt humors" of the disease.

Fumigation as practiced in olden times, with the patient, or rather the victim, inclosed in a chamber and half suffocated with the vapors of mercury and various balsams and other "perfumes," was a combination of mercury treatment by inhalation and cutaneous application; as practiced in more recent years, by placing the patient in a sort of tent with the head outside, it is almost entirely a method of cutaneous application since the mercury condenses on the body in a state of extremely fine division. After this the patient is put to bed for three quarters of an hour, enveloped in the same coverings. The fumigation is repeated every day, every other day, or twice a week, according to the indication of the case.

Undoubtedly mercury may be absorbed by this method of administration, since it has been found in the urine; furthermore, stomatitis may be produced by too frequent administrations. Very probably the mercury deposited on the skin is again volatilized and inhaled, while a small part may be absorbed directly through the glands of the skin either in a state of colloidal division and suspension or after oxidation and conversion into soluble compounds. It is, however, an inconvenient and uncertain method, dangerous and quite unsuitable as a method for the prolonged treatment of syphilis.

The use of *mercurial flannels* is really a form of inhalation therapy. These flannels, well known as "Merget's flannels," are prepared by soaking them in mercuric chlorid solution and afterwards in ammonia, which precipitates the mercury in a state of very fine division. This flannel is then enveloped in lint or gauze and placed on the patient's pillow or suspended around the neck. Bordier has shown experimentally that one of these flannels, eight by ten inches, gives off every hour an average of $\frac{1}{7}$th grain of mercury and nocturnal inhalation for eight hours is said to be sufficient for therapeutic effects. Undoubtedly mercury is inhaled and absorbed by this method as shown by elimination in the urine as well as by toxic and therapeutic effects. But, as stated by Fournier, it is useless as a fundamental method for the mercurial treatment of syphilis.

Mechanism of Absorption of Mercury Administered by Inunction.—In 1888 Merget[8] expressed the opinion that mercury when administered by inunction does not penetrate the skin at all, but is inhaled and absorbed after volatilization on the skin. Since then the subject has been a matter of considerable discussion and only within recent years may it be said that carefully conducted and controlled experiments have shown that mercury can be absorbed directly through the skin, but that in the treatment of syphilis by inunction of blue ointment considerable volatilization of mercury occurs followed by inhalation and swallowing of the vapors.

It has been contended that the intercellular bridges and basement membrane of the unbroken and intact skin forms an impassable barrier to the absorption of a heavy metal, even when so finely divided as is mercury in the form of blue ointment. But following inunctions mercury has been found in the sebaceous and sweat glands, and while it is true that these are excretory, yet it would appear possible for the mercury to find its way into the lymphatics in a state of very fine division or to be picked up and transported to lymphatic and venous channels by the leukocytes. Others maintain that by means of oxidation or chemical conversion by the fatty acids and other substances of the secretions, etc., soluble compounds of mercury are produced with direct absorption through the skin.

While the exact mechanism of absorption through the skin has not been determined, I believe that it may be said, however, that absorption of some mercury does actually occur by this route. Schamberg, Raiziss, Gavron and myself found that rabbits rubbed with calomel and blue ointments and prevented from breathing air in contact with the anointed areas, succumbed to mercurial poisoning with degenerative lesions in the liver and kidneys and with considerable amounts of mercury in these organs, together with a faint trace in the brain, but with no mercury in the lungs. Furthermore, the experiments of Wile and Elliott with ointments of mercuric salicylate, the oxids and oleate have suggested that such mercurial compounds which do not vaporize readily at body temperature and yet show the elimination of mercury in the urine when applied to patients in the form of 50 per cent. ointments, are very probably absorbed in part at least through the skin. Finally Zwick[8a] in a microscopic study of the absorption of mercury from the shaven skin of animals, applied in the form of a fifteen minute rub of mercurial ointment, has shown that mercury is not deposited or absorbed from the intact epidermal layers of cells but is apparently absorbed from the infundibula of the pilosebaceous follicles and the orifices and excretory ducts of the sebaceous glands. In other words the principal depositories were the natural openings of the skin and Zwick concludes that any mercury remaining on the intact epidermis may as well be removed with benzine or carbon tetrachlorid, as advocated by Cole and Sollmann[8b] in their "clean inunction treatment," insofar as absorption is concerned. In this connection it is interesting to note that Cole, Hutton and Sollmann gained the impression, in the course of their investigations, that patients whose skin is abnormally dry tend to be hypersusceptible to mercury, perhaps because such skins are more easily fissured, or perhaps because the ointment can be rubbed more easily into the ducts when the glands are not actively secreting.

But there is no doubt at all concerning the volatilization of mercury and inhalation and swallowing of the invisible vapor when blue ointment is applied to the skin by inunction. I believe that this is the chief means of absorption not only after the application of blue ointment which is the most volatile of mercury preparations, but also after inunction with ointments of less volatile mercury as calomel, etc. Weylander[9] who has studied this subject with particular care believes that the absorption of mercury after inunction with blue ointment occurs only by this route.

Wile and Elliott have also found mercury in the urine of a nurse who had given inunctions to patients with gloved hands over several weeks, and in experiments with calomel and blue ointments smeared on waxed paper, covered with several layers of gauze and applied to the chest in such way that none of the ointment came in contact with the skin, mercury was found in the urine forty-eight hours after the application of blue ointment and ninety-six hours after application of the calomel ointment. In the experiments of Schamberg, Raiziss, Gavron and myself rabbits inhaling air in contact with rabbits rubbed with blue ointment succumbed to mercurial poisoning and mercury was found in the skin, lungs, liver and kidney; these experiments conclusively proved the absorption of volatilized mercury, but animals inhaling air in contact with rabbits rubbed with calomel ointment did not succumb and analyses of the skin, liver, lungs and kidneys did not reveal mercury. In other words, calomel is not as volatile as metallic mercury in blue ointment; consequently it is not absorbed to the same degree. For this reason inunctions of syphilitic patients with calomel ointment does not result in as prompt therapeutic effects as follows inunction with blue ointment, as shown by Cole;[10] neither does calomel ointment produce stomatitis and

salivation as quickly as blue ointment, but yet some mercury is absorbed after inunction with calomel ointment as shown both experimentally and clinically. There can be no doubt that calomel ointment is less objectionable to patients than blue ointment and it is very much hoped that means will be found for rendering it more easily dissociated and volatilized as indicated by the work of Gambier,[11] who claims to have produced a calomel five times lighter and seven and one half times more dissociable than ordinary sublimated calomel.

According to Jenkins,[12] the rate of volatilization of mercury at blood heat is insignificant and, in the inunction treatment of syphilis, mercury is absorbed directly through the skin and not by inhalation and swallowing of vapors; but the experiments of Wile and Elliott have definitely shown that mercury is volatilized at body temperature and there can be no reasonable doubt that some and, indeed, most of the mercury absorbed after application of calomel or blue ointment to the skin, is due to vaporization followed by inhalation and ingestion. Just how the mercury is absorbed in vapor is likewise a matter of divided opinion, which subject will be discussed immediately; I believe that a portion is condensed in the mouth or upper respiratory tract and swallowed with saliva while the balance is absorbed directly through the lungs by much the same mechanism as absorption occurs through the skin.

Absorption and Elimination of Mercury Administered by Inhalation.—There is no doubt regarding the fact that metallic mercury, and to a lesser extent calomel and other salts, volatize at body and even lower temperatures. As discussed above in relation to the absorption of mercury administered in inunction, fumigation or by mercurial flannels and plasters, clinical evidence and experimental results show quite conclusively that a portion of mercury is inhaled when administered by these and similar methods.

Industrial accidents have likewise shown the absorption of mercury by inhalation, as, for example, in the case of the British ship *Triumph*, which in 1810 had some mercury containers broken in her hold and resulting in the poisoning of 200 sailors, three of whom died, as well as the fatal intoxication of all birds and cattle on board. There were also the poisoning of human beings and lower animals by the fumes of mercury from a burning mine in India in 1804 and numerous similar accidents among artisans working with mercury in mirror and thermometer factories, etc.

I have already mentioned the experiments of Schamberg, Raiziss, Gavron and myself with rabbits inhaling air in contact with other rabbits anointed with blue ointment and showing mercurial intoxications with mercury distributed in the lungs, liver, kidneys, etc.; undoubtedly a portion of the mercury was inhaled. Likewise the experiments of Wile and Elliott have been discussed above on the volatilization of metallic and various salts of mercury and the effects produced by inhalation of these vapors. In addition it may be stated that the experiments of von Baerenspring[13] showed that rabbits exposed for one quarter to an hour to the vapor of boiling mercury died within a few days and in one instance, droplets of mercury were found in consolidated areas in the lungs; rabbits kept in a cage with an open dish of mercury became ill in two weeks and died in three weeks. Similar results were observed by Eulenberg, Merget, Skudro, Ricker and Hesse;[14] Solles[15] and Binz[16] cite additional experiments of similar nature and results of earlier investigators.

Recently, however, the experiments of Cole, Gericke and Sollmann[17] have shown in their opinion that in human subjects the inhalation of mercury has no advantage over oral administration but, on the contrary, has the disadvantages of indefinite dosage and special dangers from irritation of the respiratory organs. These investigators administered from 5 to 80 mg. of calomel at a dose, totaling 225 mg. in two weeks, to each of five patients with active syphilitic lesions, by vaporizing the mercury in a strong glass tube about 10 inches long and bent to an angle of about 130 degrees, open at both ends and heated lightly in a Bunsen flame, the patient inhaling the vapor as it was formed by deep inhalations from one end of the tube. A second series of six patients were treated with inhalations of from 5 to 160 mg. of mercury rubbed up in chalk, totaling from 225 mg. in two weeks to 750 mg. in three weeks. In neither series were therapeutic results observed and the calomel proved more irritating than the metallic mercury.

Gutman,[18] however, in a series of experiments with guinea-pigs has amply confirmed the results of earlier investigators and has shown that the inhalation of the vapors of metallic mercury not only produced clinical evidences of intoxication, but also histological changes in the different organs, as well as the deposition of mercury not only in the lungs but in the liver, kidneys and other organs. Gutman has very properly called attention to the fact that vaporization of calomel or other salts of mercury may prove irritating by reason of chlorin or other gases, whereas vaporization of metallic mercury is free of irritating effects unless a concentrated vapor is inhaled.

Without at this time entering into a discussion of the practical aspects of inhalation therapy in the treatment of syphilis, there can be no doubt regarding the fact that mercury is absorbed after inhalation of vapors. In many of the industrial accidents and animal experiments referred to above, it was likely that some mercury was swallowed and probably a portion was absorbed through the skin in some instances at least; but aside from these routes of absorption, the evidence in favor of the absorption of mercury in the air passages may be regarded as conclusively proven.

There is, however, considerable difference of opinion regarding the mechanism of absorption and the practical value of inhalation methods in the treatment of syphilis; the former may, therefore, be discussed at once while the latter will be considered in a later chapter.

Mechanism of Absorption of Mercury after Inhalation.—In the first place does mercury inhaled as a vapor reach the alveoli or deeper tissues of the lungs, or is it condensed and deposited in the nose, mouth, pharynx and upper respiratory tract? I believe that the early experiments of Merget and von Baerenspring who found mercury in the lungs of rabbits exposed to the vapors of mercury, as well as those of Schamberg, Raiziss, Gavron and myself who found mercury in the lungs of rabbits inhaling vapors from the skin of rabbits anointed with blue ointment, and the experiments of Gutman who not only found mercury in the lungs of guinea-pigs by chemical analyses but demonstrated mercury in these tissues by having the animals inhale iodin immediately after the inhalation of mercury with the deposition of mercuric iodid—show quite conclusively that some mercury at least reaches these deeper tissues. Insofar as chemical analyses of the lungs are concerned, it is of course possible and indeed probable that some of the recovered mercury was from the blood; but aside from this possibility, I do not personally doubt that some of the vapor may actually reach the alveoli. Indeed, the rapidity with which mercury is ordinarily absorbed after the inhalation of mercury vapors, as judged both by clinical effects and analyses of the blood, indicates in itself in my opinion that the absorbing area must be greater than that represented by the upper respiratory passages together with the small amounts that may be absorbed after ingestion.

Granting that mercurial vapors may reach the deeper tissues of the lungs, how does absorption occur? Is it possible for the mercury to pass directly through the two layers of endothelium into the capillaries or is it first condensed and later absorbed? These questions have never been satisfactorily answered. According to Hermann,[19] Furbringer[20] and others, the mercury is first deposited since only soluble gases are believed capable of passing into the blood. Cole, Gericke and Sollmann state as their belief that most of the mercury is deposited in the upper air passages and that if any reaches the alveoli it is first deposited there before absorption; Gutman states that the vapor may be absorbed direct without primary deposition. Certainly there is no valid reason for believing that all of the inhaled mercury is deposited because at body temperature mercury is known to be volatile in the presence of moisture. As stated above, this point has never been definitely settled, although Merget's experiments with the lungs of frogs indicates that the

vapor may be absorbed direct into the blood since passage was so rapid as to be described as "instantaneous."

Neither do we know how mercury deposited in the nose, trachea bronchi and alveoli is absorbed; in the lungs doubtless a portion may be picked up by phagocytes just as particles of coal and other inorganic dusts are known to be carried into the interstitial tissues and glands Whatever the mechanism may be, it is similar to absorption through the skin. Personally, I believe a portion may be absorbed directly into the blood in a state of extremely fine division; possibly a portion, the major portion, is oxidized and by other chemical changes converted into soluble compounds which are readily absorbed. The absorption of mercury through the air passages is, therefore, a rapid process and doubtless accounts in large part for the successful administration of mercury by inunctions with blue ointment. The mercury inhaled in the form of a vapor may be in part absorbed direct without condensation; a part of that which is condensed may still be absorbed direct or after being rendered soluble by oxidation and other chemical processes which are not as yet understood.

Absorption and Elimination of Mercury Administered by Injection.—A distinct advantage of administering mercury by injection is accuracy in dosage. This is true insofar as it relates to the deposition of a certain dose in the subcutaneous or intramuscular tissues or directly into a vein or the subarachnoid space of the meninges, but when injected into the tissues it does not necessarily follow that absorption will occur in a regular and uniform manner and with all methods of injection the degree of dissociation of the active mercury ions depends upon the composition of the compound injected. In other words, the injection of a full or half complex organic compound with very firm union of mercury with the carbon nucleus may be followed by elimination of a large proportion of the dose in an unchanged form and consequently the compound may be relatively non-toxic and without much therapeutic activity. Similarly the intramuscular injection of metallic mercury in the form of gray oil (*oleum cinereum*) is followed by irregular and slow absorption, so that while the injection of mercury may readily enough satisfy the physician and patient that a certain dose has been given, it does not necessarily follow that absorption and therapeutic activity are under similar control unless care is taken in the choice of compound injected to avoid irregular and cumulative effects.

Absorption and Elimination of Mercury after Intravenous Injection.—A large number of soluble mercurial compounds—inorganic and organic—have been advocated for intravenous injection in the treatment of syphilis. In some instances insoluble compounds in colloidal suspension have likewise been injected. Of course the question of absorption does not enter here, insofar as absorption into the blood is concerned. Just what proportion of the injected dose reaches the foci of syphilitic infection and becomes therapeutically active is, however, a different matter and varies with the chemical composition of the drug. For example, organic compounds of the half or full complex variety are likely to be largely eliminated before dissociation of the mercury ion can occur.

Elimination after intravenous injection is ordinarily a rapid process, mercury being sometimes found in the urine within an hour after the injection of such inorganic salts as mercuric chlorid or the red mercuric iodid in doses varying from $\frac{1}{3}$ to $\frac{1}{6}$ grain. Elimination is usually complete within forty-eight to seventy-two hours, 75 per cent. or more of the injected mercury being recovered in the urine and feces in these periods of time; for this reason it has been found necessary to repeat intravenous injections of mercury at intervals

of one to three days when this route of administration is employed in the treatment of syphilis.

Absorption of Mercury after Subcutaneous and Intramuscular Injections of Water-soluble Compounds.—A very large number of soluble compounds of mercury have been likewise advocated for subcutaneous or intramuscular injection, since mercuric chlorid, among the first employed, was found to be quite irritating. Among the insoluble compounds, metallic mercury in the form of gray oil, calomel and mercuric salicylate have been mostly employed.

The water-soluble salts are of course more rapidly absorbed than the insoluble; in the case of both classes of compounds, however, the degree of absorption is influenced by the degree of inflammatory changes induced by the drug in the tissues, just as the rapidity of absorption of the organic arsenical compounds is influenced by this factor. Furthermore, with the insoluble compounds the degree of absorption is influenced by the kind of compound injected, being very slow in the case of gray oil and most rapid with the salicylate. The rapidity of absorption is likewise influenced to some extent by the menstrum employed, being more rapid when the compound is suspended in vegetable and animal oils than in mineral oils.

With both soluble and insoluble compounds the rate of absorption is likewise influenced to some extent according to the site of injection, being more rapid from the muscles than from the subcutaneous tissues or between the sheaths of muscles. Furthermore, mercury deposited in muscles subject to active exercise and pressure is probably more quickly absorbed than from muscles less physically active.

Lomholt has studied the rate of absorption and elimination of benzoate of mercury by injecting two patients intramuscularly with 0.1 gm. once a week; since the compound contained 43.5 per cent. mercury, this dose represented the injection of about 0.04 gm. of pure mercury. On the day following an injection, from 0.005 to 0.01232 gm. of mercury was eliminated. During the balance of the week the elimination gradually decreased to about 0.001 to 0.002 gm. on the sixth or seventh days. Elimination in the feces was more irregular and only about 21.4 per cent. of the total eliminated was found in the feces. During the period of thirty-four to forty days of treatment (five to six injections), about 35 to 48 per cent. of the total mercury injected was recovered in the urine and feces showing that about 50 to 60 per cent. was retained for longer periods of time.

Lomholt also injected a rabbit intramuscularly six times at short intervals during a period of twelve days with a dose of 0.75 c.c. of a 2 per cent. solution of the benzoate (corresponding to 0.0063 gm. of pure mercury per dose or a total of 0.0378 gm. in six doses). During this period 0.00621 gm. of pure mercury was eliminated in the urine and feces; 0.0207 gm. was found in the muscles; 0.00794 gm. in the balance of the tissues and 0.00295 gm. lost in the experiment. This experiment showed, therefore, that about 16 per cent. of the injected mercury was eliminated during the time of treatment, while about 53 per cent. of the injected mercury was eliminated during the time of treatment, while about 53 per cent. was retained in the muscles at the site of injection and 23 per cent. in the other tissues and organs.

Ordinarily we believe that absorption is more complete from the muscles than indicated by these results, but doubtless the production of albuminates of mercury in the muscles, along with inflammatory reactions, reduce the rate of absorption and due care must be exercised against cumulative effects as is true when the water insoluble compounds of mercury are injected. Data is not available on the comparative rates of absorption of the large number of water soluble compounds of mercury that have been advocated from time to time for subcutaneous or intramuscular injection in the treatment of syphilis. Probably the rate for all is similar to that found by Lomholt for the benzoate since the majority precipitate proteins and form albuminates which are slowly soluble; the degree of local irritation, however, varies with different compounds, and in the case of mercurophen, at least, I know varies

to some extent with different lots of the same compound. Doubtless the degree of local reaction influences absorption, but not to the extent of requiring serious consideration in relation to the treatment of syphilis.

Judged mainly on the basis of toxicity tests among the lower animals absorption of mercury from the muscles is somewhat more rapid than from the subcutaneous tissues; clinical observations also confirm this statement in relation not only to the soluble and insoluble compounds of mercury but to drugs in general. Absorption from fatty tissues is particularly slow and necrosis is more likely to follow than when mercury is deposited in fibrous tissue or muscles.

Absorption of Mercury after the Intramuscular Injection of Water-insoluble Compounds.—In this connection most interest is placed in the intramuscular injection of gray oil, calomel and mercuric salicylate in vegetable or animal oils.

In 1915, Schamberg, Raiziss and myself[21] studied the rate of absorption of these compounds from the muscles of rabbits and found that 40 to 50 days later as much as 46 to 48 per cent. remained inabsorbed while 71 per cent. was recovered 21 days after injection.

Lomholt found that the salicylate of mercury was absorbed and eliminated more rapidly than calomel and metallic mercury in gray oil in both human subjects and rabbits. He states that this is due to the fact that the salicylate is very difficult of dissociation and that it may be absorbed and eliminated unchanged; needless to state this is a factor of great importance in relation to toxicity and the treatment of syphilis, as I shall discuss with more detail in later chapters. Lomholt also administered these compounds to syphilitic patients and analyzed the urine and feces for mercury to arrive at the approximate amounts eliminated and retained and found a retention of 50 to 90 per cent. after 4 to 6 doses at intervals of 5 days.

The elimination of mercury after the salicylate injections was irregular and after the period of ten days from 50–60 per cent. of the injected mercury was apparently retained; elimination after the calomel injections was more regular, but at the end of ten days about 75 per cent. had been retained. The curve of elimination after the gray oil injections was very surprising; during the first fourteen days only small amounts were eliminated (less than 0.001 gm. per day), when a sudden increase occurred with the elimination of 0.005 gm. per day for more than six days. It will be noted, however, that during the entire period of eighteen days, only 9.6 per cent. of the injected mercury was eliminated in the urine and feces leaving about 90 per cent. in the tissues and largely in the muscles where the injections had been given. Of interest in this connection is the statement that some months later the patient suddenly developed severe mercurial intoxication while engaged in hard labor and without receiving any additional treatment. This indicated that the mercury had probably been encapsulated in the muscles, with sudden absorption probably the result of scattering of the "depots" as a result of muscular movements.

Even more instructive information has been secured by Lomholt in experiments with rabbits injected intramuscularly with these three drugs; it was observed that approximately only 30, 10 and 5 per cent. of the mercury injected as the salicylate, calomel and gray oil, respectively, was eliminated during the ten to eighteen days of observation, while about 35, 55 and 75 per cent., respectively, was retained in the muscles at the sites of injection. These experiments showed that the mercury of the salicylate is eliminated most rapidly and the metallic mercury of the gray oil least, while that of calomel is intermediate. In the experiments of Schamberg, Raiziss and myself described above, the salicylate was likewise found to be much more rapidly absorbed than either calomel or gray oil, although the difference in the rate of absorption of the last two drugs was not as great as observed by Lomholt.

Fournier[22] states that Oudin and Barthélemy made roentgenograms of gray oil injections and observed that after the fourth day scarcely any traces of the metal remained in the muscles. In a recent and admirable study of the same kind by Cole, Littmann and Sollmann,[23] these results were not confirmed. The authors concluded that gray oil injections are absorbed so slowly and with such danger of cumulative effects, that they should be abandoned in the treatment of syphilis as inefficient and dangerous; the salicylate was regarded as best adapted for intramuscular injections. In this connection it should be stated that soluble salts of mercury did not throw shadows on photographic plates and that their absorption could not be studied by this method.

Mechanism of Absorption of Water-soluble and Insoluble Compounds of Mercury after Injection.—The majority of the soluble salts of mercury pre-

cipitate proteins and doubtless some degree of precipitation occurs when these are injected subcutaneously or intramuscularly. This precipitation bears no relation to the amount of metallic mercury but on the nature of the compound—that is, whether organic or inorganic—and the strength of the acid from which a particular salt is derived. These factors operate by influencing the degree of dissociation or ionization of the mercury and no chemical reaction can occur while the mercury is in a non-ionized state. For example, mercuric chlorid and mercury sulphate being salts of inorganic acids ionize and precipitate proteins more actively than such salts of the weaker organic acids as the acetate, oxycyanid, cyanid, and cacodylate of mercury, although both of the inorganic salts mentioned contain less mercury than one of these salts of an organic acid, namely, the oxycyanid. Furthermore, mercuric chlorid containing 74 per cent. mercury precipitates less than the sulphate containing 67 per cent. and of the salts of the organic acids, the oxycyanid containing 83 per cent. mercury is hardly more active in precipitating proteins than the cacodylate with but 20 per cent. mercury.

Mercurophen and the succinimid of mercury do not precipitate proteins at all, except in very concentrated solutions (1:100) and it is highly probable that these and other salts of the organic acids are somewhat more quickly absorbed than mercuric chlorid and other salts of the inorganic acids. However, all of these soluble compounds probably produce some precipitation of the proteins of the tissue juices and plasma upon subcutaneous and intramuscular injection with the production of water insoluble albuminates.

It is not definitely known, however, how these albuminates or other water-insoluble compounds of mercury, as the metal itself (gray oil), calomel and salicylate, are absorbed. All produce some tissue necrosis and exert a positive chemotaxis upon polymorphonuclear leukocytes and other wandering cells; it is commonly believed that these cells pick up particles of the mercurial compounds and transport them to the blood and lymph and largely in their nuclei. In addition, it is highly probable that chemical processes result in the gradual production of soluble compounds which are absorbed into the lymph and blood. Oxidation doubtless plays some part in the production of these soluble compounds and especially in conversion and absorption of metallic mercury; since the mercury in gray oil is deposited in a mass, its conversion into soluble compounds and absorption is much slower than occurs in the respiratory tract and skin following the administration of metallic mercury by inunction or inhalation of vapors.

Physical State of Mercurial Compounds in Relation to Absorption and Elimination.—It is quite evident, therefore, that both the physical state and chemical composition of the various soluble and insoluble compounds of mercury employed in the treatment of syphilis bear an important relation to the rapidity of absorption and elimination and, as will be discussed in succeeding chapters, an even more important relation to toxicity and therapeutic activity.

By physical state I refer both to the divisibility or volatility of the mercurial compound at body temperature and the degree of divisibility at the time of administration. For example, while all mercurial compounds may be said to possess some degree of volatility at body temperature, metallic mercury is most volatile and consequently best adapted for administration by inunction or inhalation. But the degree of divisibility at the time of administration also exerts an influence; for example, finely divided metallic mercury in ointment is more volatile than the same amount in mass and it is partly for this reason that the ingestion of metallic mercury is followed by very much less absorption than when the same amount is finely divided and smeared on

the skin or incorporated in flannel or with chalk and hung around the neck. It is obvious, however, that the degree of division and facility for volatilization do not play any important part in the rate of absorption when mercurial compounds are administered by injection; under these circumstances, chemical processes are far more important, and especially oxidation with the conversion of mercurous to mercuric compounds and other chemical reactions concerned in the production of soluble compounds.

While the rate of decomposition or ionization of the compounds into the mercurial ions apparently influences the production of albuminates and these in turn the rate of absorption, yet ionization is not nearly so important in relation to the absorption of the compound as it is to toxicity and therapeutic activity. The salicylate of mercury, for example, is a pseudo- or half-complex compound, in which only one of the two valencies of the divalent mercury is connected to the carboxyl of the acid, the other being connected directly to the carbon of the benzene nucleus; as a result, the salicylate is less easily decomposed than some other compounds and its lower toxicity appears to be due to this slow dissociation or ionization. For this reason some of the salicylate may be excreted unchanged. This factor may also influence the rate of production of albuminates in the tissues and these in turn may influence the rate of absorption, although the importance of this factor is not to be emphasized, since the albuminates are readily absorbed, as shown by the rapid absorption of those formed by the soluble salts of the inorganic acids as when mercuric chlorid is injected subcutaneously or intramuscularly.

Distribution of Mercury after Absorption.—It is highly probable that mercury is distributed in the blood in the form in which it is absorbed and, as stated above, this is influenced to some extent by the ionization capacity of the compound. Metallic mercury is first oxidized and mercurous salts are converted into mercuric compounds; with the ionizable compounds albuminates are now produced and most investigators have affirmed that the mercury circulates as such. After intravenous injection it is believed that the same changes occur. In the case of compounds which do not ionize at all as the full complex organic compounds, or, those that ionize partially as the half-complex and pseudocomplex compounds, as mercuric salicylate, it is believed that a portion may be absorbed unchanged and be excreted as such. It is difficult to understand, however, the form in which such a compound circulates, unless in colloidal suspension or within the leukocytes.

Lomholt states in this connection that his analyses have shown mercury in the leukocytes and while the amounts were relatively small, owing to the small numbers of leukocytes in the blood, yet the amount per leukocyte was large. It is likewise possible that metallic mercury may be absorbed through the skin and alveoli of the lungs in a state of extremely fine division and be transported in colloidal form or after being picked up by the leukocytes and thereby carried in the blood. Lomholt has also found mercury in the erythrocytes and plasma, mostly in the latter; fractional precipitation of the serum with ammonium sulphate showed equal amounts in the albumin and globulin fractions. According to this investigator the saturation point of the blood is about 0.003 gm. per 1000 c.c.; when this amount is exceeded elimination occurs. Rosenbloom[24] found as much as 0.012 gm. per liter of blood in a case of acute poisoning.

After reaching the lymph and blood, mercury is widely distributed throughout the body and is found in almost every organ including the spleen, lungs, intestine, buccal mucosa, brain, muscles and bones. Lomholt states that it is transmitted through the placenta to the fetus and this is well substantiated by clinical experience. He also found traces in ascites fluid,

which is to be expected when collections occur at a time when mercury is present in the blood. Larger amounts are found in the kidney, intestinal wall and liver than elsewhere; considerable amounts are usually deposited in the liver. This distribution occurs in both acute poisoning and during prolonged administration regardless of the route of administration. According to Lomholt, the kidneys contain the highest concentration, namely, 0.0016 gm. per 100 grams in a treated case, and 0.017 gm. per 100 grams in a poisoned case. The liver was next in percentage content and first in actual content among the internal organs, owing to its large size. The intestinal walls contain the next largest amount in point of percentage. It is highly probable that high concentrations also occur in syphilitic lesions and especially those involving bone; of interest in this connection was the finding by Mastin and myself[25] of an enormous number of globules of metallic mercury in the tissues of aneurysm of the descending aorta involving the dorsal vertebrae. As early as 1579, Fernel[26] states in his work on the venereal disease that metallic mercury may be found in the "putrid caries of bones" if the patient has been exposed to the vapor of mercury or of cinnabar, and also in those who have had repeated rubbings with the ointment. Since then, several observers have recorded the presence of metallic mercury in the necrotic bones of syphilitics receiving vigorous mercurial treatment; the literature of the last twenty-five years, however, contains but few records, owing probably to the practice of less vigorous dosage with mercury in the treatment of syphilis. In our case we could not ascertain from the patient the form in which mercury had been taken. A second aneurysm of the first portion of the descending aorta, but not involving bone, did not show the presence of these globules of mercury.

Mercury in the Brain and Cerebrospinal Fluid.—As stated above traces of mercury have usually been found in the brain. This occured in the rabbit experiments of Schamberg, Raiziss, Gavron and myself following inunctions with blue ointment and various observers have found traces by chemical analyses in the brains of the lower animals treated in various ways with mercurial compounds, as well as in the brains of human subjects succumbing to mercurial intoxication. These findings are of considerable interest in relation to the treatment of neurosyphilis, as are the results of studies on the presence of mercury in the cerebrospinal fluid. Wile and Elliott[27] found none in the spinal fluid of patients after inunctions with blue and calomel ointments, and following injections of the salicylate, succinimid and bichlorid. In no case were they able to detect the faintest trace by Elliott's method. Believing that perhaps the amount of fluid tested was too small, they examined the fluids from several different cases that had been taking mercury in various forms for a long period of time. The results were likewise negative. They were able, furthermore, to test the fluid of a patient who died of mercurial poisoning from the ingestion of bichlorid; these results were also negative. Lomholt, however, claims to have found approximately 0.0004 gm. per liter in the spinal fluids of treated cases. Others have also claimed that traces are to be found after prolonged administration or in acute bichlorid poisoning, but the matter of penetration of mercury to the cerebrospinal fluid is still undecided. It is sometimes stated that spinal drainage after the method of Dercum and Gilpin, which consists in the removal of spinal fluid until the flow is reduced to only a few drops per minute, at a time when the patient is under vigorous mercurial treatment, results in an increase of mercury reaching the tissues of the cord and brain and the spinal fluid. These effects are suggested by similar results in the penetration of arsenic, which is also a heavy metal, during treatment with arsphenamin or its substitutes; likewise

by signs of improvement in the spinal fluid and the patient. But actual proof that the penetration of mercury is increased by this procedure cannot be said to have been furnished by chemical analyses of the spinal fluid. It is also possible that intraspinal injections of mercurialized serum or other substances may increase the penetration of mercury to these tissues and the spinal fluid, if mercury happens to be in the blood, by producing localized areas of passive hyperemia with extravasation of plasma (the so-called "aseptic meningitis"), but definite proof in the form of chemical analyses for mercury under these conditions has not been furnished.

The subject has not, however, attracted the attention it deserves in relation to the treatment of neurosyphilis. Mercury dissolved in serum has been injected directly into the spinal subarachanoid space in the treatment of neurosyphilis, but this subject will be discussed in a later chapter.

Retention and Elimination of Mercury in Relation to the Treatment of Syphilis. *Storage or "Depots" of Mercury.*—The elimination of mercury in the urine and feces has already been briefly alluded to in this chapter in relation to the administration of mercury in different forms and by varying routes. In the treatment of syphilis absorption usually proceeds more rapidly than elimination, which results in storage, at least for a time. If the administration is moderate, a state of equilibrium is gradually reached during which excretion may keep pace with absorption. If absorption exceeds excretion beyond a certain point, symptoms of toxicity may appear, which, however, in mild cases tends to gradually disappear if absorption ceases or is reduced while elimination is maintained. The problem in the treatment of syphilis is, therefore, to choose the compound, dose and route of administration which will result in the maximum degree of storage without injury, on the basis that the best therapeutic results will follow when absorption and elimination are so adjusted that the maximum non-toxic amounts of mercury are available in the blood and infected tissues over a long period of time. Lomholt has calculated that the blood may carry from 0.001 to 0.003 gm. of mercury per liter without producing toxic tissue changes and symptoms and that the tissues of an adult may store from 0.090 to 0.150 gm. without toxic effects after about forty inunctions.

As previously stated, however, mercury tends to accumulate in the tissues under all forms of administration; this tendency is least marked with intravenous injections of the soluble salts and most marked following intramuscular injections of gray oil. Among the other insoluble salts commonly employed in the treatment of syphilis, the salicylate is less likely to accumulate than calomel, because a portion of the salicylate may be absorbed and eliminated unchanged without as much tendency to form combinations with the tissue proteins and thereby become stored.

Therapeutic Activity of Stored Mercury.—An important question now arises: is the stored mercury therapeutically active? As discussed in a preceding chapter, it does not appear that stored arsenic from arsphenamin or its substitutes is active because of continued oxidation to relatively inert pentavalent compounds. But I am convinced that stored mercury is therapeutically active and that due consideration should be given this factor in our treatment of *chronic* syphilis. My opinion, however, is based entirely upon clinical results and not upon actual animal tests with emulsions of mercury laden tissues. I have commonly observed that, after a course of mercury, the complement-fixation test may still yield a positive reaction and lesions may still be present in the skin, periosteum, etc., but that after a rest of weeks or months without treatment the serum reaction becomes gradually weaker and the lesions continue to heal. I believe that it is a com-

mon mistake in the treatment of *chronic* syphilis to "push" treatment too hard and largely because the Wassermann reaction remains positive; I have learned that to give courses of treatment of four to six weeks' duration, followed by periods of three to four months of rest, yields best results in the treatment of *chronic* active and *especially of chronic latent syphilis;* one is frequently surprised with the results to be secured by what may be called mild, but persistent efforts of this kind even in the so-called Wassermann-fast cases; but I shall discuss this matter with more detail in chapters devoted to the treatment of chronic syphilis. Here it may be stated, however, that the problem is entirely different in acute early syphilis; it is my opinion that mercury has much less value in this stage since it does not work "fast enough" and that preference should always be given arsphenamin or its substitutes unless a contraindication exists to their administration.

Equilibrium between the Absorption and Elimination of Mercury.—As stated above, the ideal administration of mercury may be said to call for rapidly storing in the tissues the maximum amount free of producing tissue injury and toxic effects, and, according to Lomholt, maintaining from 0.001 to 0.003 gm. per liter in the blood over periods of say four to six weeks at a time.

For this purpose *intravenous injections* of soluble salts may be acquired every day or every other day, since the dose must be small and elimination is rapid with slow storage or accumulation.

Intramuscular injections of the ordinary soluble salts would appear to be required about twice weekly in doses of ¼ to ⅙ grain according to Lomholt's observations with the benzoate and about 50 per cent. of the mercury is retained and stored. Intramuscular injections of the salicylate are apparently required at least in the first part of a course, about every five days in dose of 1 grain, since it is the most rapidly eliminated of the insoluble salts; 55 per cent. tends to accumulate, which is less than with calomel or gray oil. As accumulation occurs, the intervals between doses should be gradually lengthened. Calomel in dose of about 1 grain should be given every 5–7 days, since it is more slowly absorbed and eliminated, about 75 per cent. being retained and stored. For this reason the intervals should be likewise gradually lengthened instead of being kept uniform. Gray oil in dose of about 1 grain of mercury may be given at first every 5 to 7 days but, because it shows the greatest tendency for accumulation (90 per cent.) with only about 10 per cent. elimination, the intervals between injections must be gradually lengthened to avoid sudden or progressive toxic effects even after a course of injections has terminated.

Absorption and elimination after inunctions of blue and calomel ointments is more difficult to gauge as likewise, after ingestion of the protiodid or bichlorid of mercury since absorption is apt to be more irregular. It is well known, however, that storage occurs; probably about 50 per cent. of mercury is stored after inunctions with blue ointment so that, while daily rubbings with 1 to 3 drahms may be permissible for the first week or two, the dose and frequency should be then reduced in order to avoid over storage and toxic effects.

It is readily understood, therefore, that the administration of mercury in the treatment of syphilis should take into consideration these very important factors of absorption, storage and elimination and I shall discuss the subject in this relation with more detail in chapters devoted to treatment. As far as possible an effort should be made to maintain a balance between absorption and elimination, and the studies of Lomholt have aided us greatly in the practical administration of various well known mercurial compounds in the treatment of chronic syphilis.

In practice, however, this ideal of equilibrium between absorption and elimination is very difficult to attain, because the degree of absorption is never known unless the mercury compound is injected intravenously and elimination through the urine and feces requires intricate and laborious chemical analyses; furthermore, as stated above, it is likely that some mercurial compounds are eliminated unchanged in which the medicament may have exerted no therapeutic effects. At best we can obtain only approximate results by careful consideration of the known facts concerning absorption, elimination and retention of mercury in relation to different compounds employed in the treatment of syphilis and in relation to the different possible routes of their administration, as well as by careful observation for the clinical evidences of tissue injury, poisoning and therapeutic results.

Just how long elimination continues after cessation of treatment is difficult to state and varies, of course, according to the kind of compound employed, the dose and route of administration, the duration of treatment and the condition of the two main enunctories, namely, the kidneys and liver. After the intravenous injection of the soluble compounds elimination is most rapid and slowest after the injection of metallic mercury in gray oil.

Elimination of Mercury in the Urine, Bile and Feces.—The largest amounts of mercury are eliminated in the urine. Lomholt has estimated that from ⅔ to ⅚ of the total eliminated escapes by this route. Elimination in the urine is usually regular and uniform although, as previously stated, it varies according to the kind of compound and route of administration. After the intravenous injection of soluble salts, mercury may be found in the urine within an hour and is rapidly eliminated. After the intramuscular injection of soluble salts, mercury is generally found in the urine in twelve to twenty-four hours, as likewise after the inhalation of vapors. After inunction with blue ointment mercury may be found in the urine in twenty-four hours, absorption and elimination being usually later, however, after inunctions with calomel.

Elimination of mercury through the kidneys usually produces a diuresis, which is sometimes made use of in the treatment of cardiac dropsy. According to Lomholt, there does not appear to be a uniform relation, however, between the quantity of urine excreted and the mercury content, but the elimination of very large amounts of mercury always seems to be accompanied by the passage of large amounts of urine. Roughly from 65 to 80 per cent. of all mercury eliminated is excreted by the kidneys in the urine.

While excretion in the urine parallels absorption to a certain degree and while there is no limit to the amount of mercury that may be absorbed, there is a distinct maximum limit to excretion by the kidneys without damage to these organs which Lomholt has estimated at about 0.009 gm. per day; when this amount is exceeded, tissue injury may result.

Nothing is known of the chemical form in which mercury occurs in the urine; with the half and full complex organic compounds it is possible that portions may be eliminated unchanged.

Elimination by the bile and intestines rank next in importance. Lomholt has found from 0.0075 to 0.0165 gm. per liter of bile of animals. Approximately 20 to 35 per cent. of the total elimination of mercury occurs in the feces. It is entirely likely that a portion eliminated in the bile is resorbed lower down in the intestinal tract with a repetition of the cycle of absorption and elimination and accounting in part for prolonged elimination after cessation of administration.

Mercury has been found in the pancreas of animals treated with various compounds and likewise in the pancreas of fatal cases of bichlorid poisoning of human beings; it is probable, therefore, that mercury may be stored in this organ during the treatment of syphilis and that a portion may be excreted with the pancreatic secretions, but this has not been proven by direct experimental investigation.

Elimination through the intestinal walls, and particularly by the colon, has long engaged a great deal of attention since pathological changes are so prominent in the mucosa in cases of poisoning. Chemical analyses have shown mercury in the intestinal walls and it is generally believed that excretion occurs through the glands of the mucosa. Since mercury is also excreted by the buccal mucosa, or, at least, may be present in the gums just as may occur in lead and bismuth poisoning, it is highly probable that some mercury is eliminated by these channels. However, it is also possible and indeed probable, that some of the mercury found by analysis in the intestinal walls is due to absorption into the lymph channels of mercury eliminated in the bile or after ingestion and that the necrotic changes in the mucosa of the colon may be produced by the local irritation of mercury in the intestinal

contents. The investigations of Lomholt have shown that while elimination of mercury in the urine usually fluctuates with regularity according to the amount of mercury administered, elimination in the feces shows no regularity, but is quite irregular, varying from day to day in an unaccountable manner. Lomholt also noted that the quantity and consistency of the feces bore no relation to the mercury content although the larger amounts were found in feces of soft or fluid consistency.

Wile and Elliott have found traces of mercury in the stomach washings after several inunctions with blue and calomel ointments. Whether the mercury was eliminated by the mucosa or swallowed in the saliva, could not be determined; personally I believe that the latter was probably the case since available data indicates that while both mercury and bismuth are eliminated in the mouth, lower ileum and first portion of the colon, that elimination does not occur in the stomach.

Nothing is definitely known of the chemical form in which mercury is eliminated in the bile and gastro-intestinal mucosa. After reaching the intestinal contents albuminates are formed and in the colon, sulphids may be produced in addition. Of course when metallic mercury and its salts are administered by ingestion, portions may be passed unchanged. Likewise it is possible that half and full complex organic compounds administered by injection may be eliminated in part unchanged, owing to failure of dissociation in the blood and tissues. Otherwise it is commonly believed that mercury in the feces occurs as the insoluble albuminates and sulphids.

Elimination of Mercury in the Milk, Saliva and Perspiration.—In 1900 Legales and Duperoy[28] stated that mercury could be found in the milk of women who were being treated with mercurial compounds, and in amounts sufficient to be of value in the treatment of syphilitic infants. Clinical evidence abundantly supports these findings since it is well known that mercurial treatment of syphilitic mothers favorably influences the disease in their syphilitic children. Recently Wile and Elliott have also found mercury in the milk of several nursing women when tested one week after treatment with inunctions; to avoid the possibility of the milk being contaminated by the skin, injections of bichlorid were given and mercury was found in the milk forty-eight hours later.

Traces of mercury have likewise been found in the saliva and perspiration. Elimination by the salivary glands has, of course, been long accepted largely on the grounds that salivation is such a prominent sign of mercurial intoxication. But actual analyses of the saliva have usually shown that only traces may be present and, in some instances at least, the recovered mercury may have been due to condensation and deposition of mercury vaporized from the body during inunction treatment rather than as a result of excretion by the glands. For example, I believe that this possibility existed in the analyses reported by Wile and Elliott, who found mercury in the saliva of patients after inunctions with blue and calomel ointments and the dressing of a syphilitic ulcer with wet applications of bichlorid, without any evidences of stomatitis or salivation.

Mechanism of Therapeutic Activity of Mercury in Syphilis.—That mercury is therapeutically useful in syphilis is abundantly substantiated by centuries of clinical experience. Doubtless cures have been effected in many cases although the number of these as gauged by the modern criteria of more extensive clinical knowledge of the lesions of chronic and latent syphilis combined with the Wassermann reaction of the blood serum and spinal fluid, roentgenologic examinations, etc., is not as large as was believed ten to fifteen years ago. Undoubtedly mercury may dissipate the obvious lesions of syphilis and, when taken over long periods of time according to Fournier's methods, the famous "chronic intermittent treatment," may maintain a state of freedom from obvious lesions and symptoms for many years. But actual and complete cure in the sense of complete eradication of all spirochetes from the tissues is of doubtful occurrence; certainly this result cannot be brought about by treatment with mercury alone in the majority of cases and especially when treatment is begun after the first year of infection.

Just how mercury brings about curative effects in syphilis is not definitely known; the subject is discussed with more detail in Chapter XXIV. Lomholt is of the opinion that not enough mercury can be maintained in the tissues and blood without toxic reactions and over sufficiently long periods of time, to bring about direct spirocheticidal effects in human syphilis, since the experiments of Kissmeyer have indicated that spirochetes may be cul-

tivated in horse serum containing as much as 0.020 gm. of mercuric chlorid per liter.

In his opinion mercury acts by stimulating the tissues to produce spirocheticidal antibodies, but direct evidence of this is lacking, as I have already discussed in Chapter XXIV.

It is my opinion that the curative effects of mercury in syphilis are due to the direct spirocheticidal effects of the mercury ions. Toxicity for the cells of the host is likewise due to the same ions, but it is possible to secure spirocheticidal effects without toxic effects through the accumulation of mercury in the syphilized or infected tissues. Both toxicity and spirocheticidal activities vary among different compounds of mercury according to the rapidity and ease with which the ions are dissociated before elimination occurs; with the salicylate of mercury and flumerin, for example, elimination of unchanged drug may occur before dissociation takes place which accounts for their lower toxicity and likewise lower spirocheticidal activity so that the chemotherapeutic indices of single doses are about the same as mercuric chlorid.

Insofar as the destruction of spirochetes in the blood is concerned, it would appear that almost the maximum tolerated doses of all mercury compounds are required for this result. Therefore, in general terms, mercury is not suitable for the treatment of syphilis in its acute, early, spirochetemic stages. And why is this the case? Because when mercury is injected intravenously it is eliminated so rapidly that the injections must be made at almost daily intervals to maintain sufficient mercury in the blood for spirocheticidal effects and while these amounts may not be toxic for such organs as the heart, pancreas, central nervous system, etc., they may be for those organs concerned in elimination as the kidneys and liver, because so much mercury is brought to them for excretion. In other words the affinity of mercury ions for *Spirochæta pallida* is undoubtedly greater than for the body cells in general, but to maintain spirocheticidal amounts in the blood over a sufficient period for curative effects may result in the accumulation of toxic amounts in the eliminating organs and especially the kidneys. Rapid elimination after intravenous injections accounts for the fact that the acute early lesions of the skin and mucous membranes may not be greatly influenced at all or only as long as the injections are given at daily or every two day intervals. For this reason I believe that arsphenamin, neoarsphenamin or their substitutes are always to be preferred in the treatment of syphilis in its acute early stages, because the toxicity of these for all body cells, including the excretory organs, is so much less than mercury in relation to their spirocheticidal amounts that sufficient amounts for curative purposes can be ordinarily maintained in the blood for longer—much longer—periods of time without injury to the excretory or other organs.

But how then does mercury exert curative effects in syphilis?—and no one can deny that this occurs. As previously stated, I believe spirocheticidal effects are due to the mercury ions and that mercury accumulates in syphilitic tissue to a greater extent than in non syphilitic tissue. In other words, I believe that mercury has a greater affinity for *Spirochæta pallida* and the inflammatory exudates engendered by it than it has for non-infected tissues, and that during the administration of mercury it tends to accumulate in the infected tissues, soon reaching a concentration sufficient for spirocheticidal effects. The finding of such large amounts of mercury in an aneurysm of a syphilitic individual by Mastin and myself, previously referred to, has greatly influenced me to the opinion that mercury tends to accumulate in syphilitic tissues; of course, a portion is eliminated, but when mercury is administered

by mouth, inunction or by intramuscular injection it is easy so to regulate the dosage that accumulation to therapeutic concentration occurs in the infected tissues without toxic effects upon the kidneys, liver and other organs.

Just how mercury kills spirochetes after being brought into contact with them is unknown. It may be that the ions united with the proteins of the parasites brings about precipitation and death, but the exact mechanism of destruction is as obscure as the general subject of the destruction of parasites by germicides.

Other Pharmacological Effects of Mercury.—Very few studies have been made on the pharmacological actions of mercury beyond the subjects of absorption and elimination because the subject has not proven of much clinical interest. Dreser[29] studied the action of various compounds of mercury on the isolated heart and more recently Mueller, Schoeler and Schrauth[30] have reported experiment on the effects of several organic preparations on the circulation and respiration in cats in which injection was made intravenously. Salant and Kleitman[31] have carried out experiments with compounds of mercury on different animals and on the isolated heart of the dog and turtle. They also studied the effect of different substances on the action of mercury, giving some attention to its absorption from the small intestine and when given by intramuscular injection. The reaction of the rabbit to mercury was qualitatively the same as that of dogs, but their experiments indicated that rabbits could tolerate larger amounts of mercury than dogs.

In brief, mercury was found highly toxic to the heart, causing various cardiac irregularities, such as heart block, delirium cordis and finally paralysis. It is also toxic to the respiration, but the effect in this case is not nearly so pronounced as on the circulation. Furthermore, the action of mercuric salts may be greatly increased by citrate or by epinephrin, suggesting that under certain conditions associated with disturbances of metabolism the toxicity of mercury may be still more increased.

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CHAPTER LIII

TOXICOLOGY OF MERCURY IN RELATION TO METHODS OF ADMINISTRATION AND THE TREATMENT OF SYPHILIS

Since mercury has been used and abused for centuries in the treatment of syphilis, it is not surprising that a great deal has been written on the toxicological effects and especially from the clinical standpoint. While threatened for a while to be displaced by arsphenamin and its substitutes in the treatment of syphilis, I believe that we may still agree with Fournier that "the position which it has attained in therapeutics after so many vicissitudes will always be maintained, for it has a powerful action on the disease—an action which has been disputed, but which is confirmed by all evidence, and cannot be ignored." But "the public of to-day bears malice against mercury in remembrance of the past" because of its toxic effects, especially stomatitis and salivation, resulting from errors in its administration, so that a thorough knowledge of its toxicological action in relation to methods of administration is almost of as much importance to-day in the treatment of syphilis as before the discovery of arsphenamin.

Mercurial Compounds of Special Interest from the Standpoint of Toxic Reactions in the Treatment of Syphilis.—Our interest, therefore, is mainly confined to the toxic effects produced by mercurial compounds employed in the treatment of syphilis, rather than to mercury poisoning in general. Naturally a very large number of various soluble and insoluble salts have been described and employed for the treatment of this disease in the search of centuries for compounds less toxic, more therapeutic and more elegant than others. Within the last ten years more systematic researches have been made along the lines laid down by Ehrlich in the development of the chemotherapy of the organic arsenical compounds in efforts to evolve a compound of reduced toxicity for the host with enhanced destructive activity for *Sp. pallida*, but so far none have been produced approaching anywhere near arsphenamin and neoarsphenamin in these respects. As a result, however, a very large number of new organic mercurial compounds have been produced, a recent excellent bibliography of the literature of which has been published by Whitmore.[1] From the standpoint of the treatment of syphilis and bacterial infections such compounds as asurol, mercurophen, flumerin, mercurochrome and mercurosol represent distinct advances, but so far no one has yet succeeded in evolving a mercurial compound possessing a chemotherapeutic index for *Sp. pallida* approaching that of arsphenamin.

As will be shortly discussed, the chemical constitution of mercurial compounds influences their local and systemic effects quite considerably; for example, mercuric chlorid and the yellow iodid (protiodid) are more apt to produce gastro-intestinal irritation than mercury with chalk, and intra-

muscular injections of the former are quite painful, because the soluble inorganic mercuric salts in general are especially irritating. It would lead me too far afield to discuss in detail even a small proportion of the large number of soluble and insoluble inorganic and organic compounds advocated from time to time for the treatment of syphilis; it is sufficient to concentrate on such compounds as the bichlorid, protiodid, succinimid, salicylate, calomel, the metal itself in various preparations and a few of the newer compounds as mercurophen, flumerin and mercurosol. In the treatment of syphilis mercury may be administered by four different routes, namely, by injection, by cutaneous application, inhalation and by ingestion; but different compounds vary in their toxic and therapeutic effects according to the route of administration, although the main lesions and systemic effects are essentially similar.

Chemistry of Mercurial Compounds in Relation to Toxicity and Therapeutic Activity.—Despite a great deal of research in the chemotherapy of mercurial compounds, no rules or definite "leads" have been discovered for establishing a relation between chemical constitution and biologic effects. Indeed, very little advance has been made in this field; it is true that new compounds of greatly reduced toxicity have been produced in the past few years, but these are likewise of reduced therapeutic activity or toxicity for the parasites. The triumph of arsphenamin was the discovery of a compound of reduced toxicity for the host with increased toxicity for the parasite, but similar results have not yet been achieved for mercury. If we could reduce the toxicity of mercury for the host without reducing at the same time its spirocheticidal activity or, if we could increase its spirocheticidal effects without at the same time increasing its toxicity for the host, a real advance would be made. Most investigators have sought to produce new compounds of lowered toxicity for the host of which flumerin is a striking example, but it is necessary to administer relatively large amounts for spirocheticidal effects leaving the chemotherapeutic index, which is the relation or proportion between the maximal tolerated and minimum curative doses, practically unchanged. In other words, compounds least toxic for the host are proportionately least toxic for spirochetes; simply because some compounds, as flumerin and mercurosal, can be given in larger doses because of reduced toxicity for the host does not necessarily mean that they are more curative in the treatment of syphilis, athough it may mean that such are less likely to produce local irritation of the muscles, gastro-intestinal tract, etc.

Therefore, we do not yet know how to increase the spirocheticidal activity of mercury while reducing its toxicity for the host or keeping this action unchanged. Stimulating and promising efforts have been made, however; for example, in 1912 Blumenthal and Oppenheim[2] reported work offering considerable encouragement along these lines, although in some respects it has not been generally confirmed by Kolle and others. Blumenthal and his associates believed that the chemical constitution of mercury compounds influenced their affinity for the tissues in that the aromatic compounds behaved in the body according to whether the mercury was bound to the radicle by one or two valences. Ionizable compounds showed, according to their experiments, a distinct affinity for the liver but not for other organs; colloidal mercury lacked this hepatotropism. Mercury bound by two valencies, as the sodium salt of di-amino-mercuri-diphenyl-di-carbonic acid, did not show any tissue affinities and was but slightly toxic and compounds in which the two amino groups were replaced by nitro or hydroxyl groups were found to circulate for some time in the blood with final localization in the liver. The amino groups, therefore, were believed to hinder the deposition

of mercury. The introduction of iodin or bromin decreased rather than increased tissue affinity. None were found to have any special affinity for the central nervous system, which lead them to believe that the curative activity of mercury in neuro-syphilis is not due to any special affinity for these tissues but rather depends upon the localization of mercury in the liver with the production there of new spirocheticidal substances. I cannot agree with these latter propositions, for there is little or no histological evidence to show that the compounds of proven curative activity in syphilis have any special affinity for the liver; on the contrary, I believe that mercury does have a special affinity for syphilitic tissue in general regardless of the organ involved.

But if we are still in the dark regarding the chemistry of mercurial compounds insofar as influencing separately toxicity for the host (organotropism) and toxicity for the parasites (parasitropism), what do we know of chemical laws by which toxicity for both host and parasites may be increased or decreased together even though we cannot greatly influence them separately?

Does solubility exert an influence? In a broad and general manner, compounds soluble in water or weak alkaline solutions are more toxic than those that are insoluble; but many of the latter are soluble in the tissues, which tends to reduce the differences. Likewise some of the soluble compounds, like the bichlorid, are more irritating to the tissues; but this is not always the case as shown by the lesser irritating effects of such soluble compounds as mercurophen, mercurochrome, flumerin and mercurosal. Solubility, therefore, is not of itself a criterion of either increased or decreased local and systemic toxicity for either host or parasite, but when considered in relation to mercury content and, more especially, the rate of dissociation of mercury ions in the tissues, solubility is a matter of importance in that soluble compounds of readily ionized mercury are more toxic than insoluble compounds of slowly ionizable mercury.

Does the content of mercury exert an influence? In other words, are those compounds containing most mercury proportionately most toxic and most spirocheticidal? In a broad and general manner, this is true as shown by the investigations of Schamberg, Raiziss and myself shortly to be reviewed, but *only when the compounds are of the same class in relation to solubility and the rapidity with which the mercury is dissociated in the tissues.* For example, the cyanid of mercury containing 79 per cent. of mercury is more toxic than the succinimid containing 50 per cent., because the former contains more mercury and because both belong to the same class of compounds insofar as solubility and the rate of dissociation of mercury in the tissues are concerned.

Does the rate of dissociation or ionization of the mercury in the tissues exert an influence? In other words, are those compounds in which the mercury is firmly bound to the organic radical less toxic than those in which the mercury is quickly dissociated? In 1911 Muller, Schoeller and Schranth[3] showed that those organic compounds in which the mercury was very firmly bound to the organic radicle, as in the case of Pesci's mercury di-benzoic acid, were much less toxic than compounds in which the mercury was less firmly bound; they stated that the effects of mercury set in as the mercury is split off and that the intensity of action depends on the readiness of this liberation and on the rapidity with which the molecular form is excreted. On this basis they divided the mercury compounds into (*a*) the inorganic, in which dissociation is rapid; (*b*) the pseudo-complex, in which dissociation is less rapid; (*c*) the half-complex, in which ionization or dissociation is still less rapid, and (*d*) the full-complex, in which each valence of mercury is so firmly bound to an organic radical, that dissociation is very slow or does not occur at all before the compound is excreted unchanged and practically inert. Therefore,

inorganic compounds would be most toxic followed in order by pseudo-complex, half-complex and full-complex compounds, the latter being almost free of toxicity or rapidly eliminated before dissociation of mercury occurs. Abelin found the aromatic compounds less toxic and Tuwtsungming[4] observed that the *local* irritation is strongest with the inorganic compounds while *systemic* toxicity is likewise highest for inorganic mercuric salts, next with the organic compounds and least with the mercurous salts, in general confirmation of the claims made by Muller and his associates; Schrouth and Schoeller[5] believed, from their studies of a large number of mercury compounds, that the therapeutically active substances are to be found in the class of half-complex compounds because in the full-complex compounds the mercury is dissociated so slowly as to render them inert and in the pseudo-complex and inorganic compounds, dissociation occurs so rapidly as to render them too highly toxic.

Kalges and Schreiber,[6] however, state that the toxicity and spirocheticidal (for *Sp. gallinarum*) effects of the mercury-dye compounds bears no relation to mercury content or rate of dissociation. Kolle and Rothermundt[7] have expressed the same opinion after experiments with "asurol," dinitro-mercuri-diphenyl-carboxylic acid, "argulan," sulfamino-antipyrene mercury, and other compounds in the treatment of hen spirillosis (*Sp. gallinarum*), stating that the therapeutic value of mercury compounds bears no relation to mercury content, rate of dissociation of mercury or solubility.

But I am of the opinion that the rate of ionization or dissociation of mercury is of considerable importance in determining both toxicity for the host and toxicity for the parasites (spirocheticidal activity). Apparently ionized mercury is equally toxic for both and compounds which are absorbed and eliminated unchanged are apt to be the least toxic and least therapeutic. Without dissociation or ionization no chemical action can occur and the compound is inert. Rapidly ionized compounds like mercuric chlorid are most toxic for the host and parasites and likewise most toxic by local contact with the tissues when injected subcutaneously and intramuscularly or applied to mucous membranes, as when administered by ingestion. The chemical properties of the ionizable mercurial compounds are largely produced by the ions they liberate on dissociation and as a consequence the physiological effects of electrolytes are due to their ionic state and through the ions that are present in a cell many of its chemical processes are controlled. Furthermore, chemical constitution may control the rate and degree of dissociation and ionization of mercurial compounds. The salicylate of mercury, for example, owes its popularity in the treatment of syphilis largely to the fact that it produces but little pain and local reaction when injected intramuscularly because of its half-complex chemical constitution, as a result of which the mercury is dissociated and ionized slowly and, indeed, a portion of the substance may be absorbed and eliminated unchanged. It is an anhydrid of ortho-oxy-mercuri-salicylic acid in which only one of the two valencies of the divalent mercury has been connected to the carboxyl of the acid. The other one is directly connected to the carbon of the benzene nucleus which results in a compound very difficult of dissociation and ionization. Of course, when injected intramuscularly, dissociation slowly occurs because toxic effects and curative activity are apparent in the treatment of syphilis, but evidently the rate of ionization is slower than in the case of calomel and probably of gray oil as well. As stated above, we have not yet discovered a means for increasing or decreasing the toxicity of either separately, but the firmness with which the mercury is bound in the molecule exerts an influence upon the rate and degree of ionization which influences both toxicity and therapeutic activity.

In my opinion, solubility in the tissues, percentage of mercury and rate of dissociation of mercury influence toxicity and therapeutic activity. The least toxic compounds are those which are insoluble and contain small amounts of mercury in which the mercury is firmly attached and almost non-ionizable; the most toxic are those which are soluble and contain large amounts of quickly ionized or dissociable mercury. Between these extremes are many compounds. Flumerin, the disodium salt of hydroxy-mercuri-fluorescein, is soluble, contains 32.5 per cent. of mercury but is at least 15 times less toxic than mercuric chlorid containing 74 per cent. of mercury; the difference in toxicity is not due to solubility, as both are readily soluble, but to the differences in mercury content and to the fact that the mercury in flumerin is less readily dissociated. Mercurosal, di-sodium-hydroxy-mercuri-salicyl-oxy-acetate containing 43.85 per cent. mercury is 7 to 10 times less toxic than mercuric chlorid, not because of solubility, since both are readily soluble but to the fact that the latter contains less mercury and because the mercury is bound slightly more firmly in the molecule.

This brief review shows, therefore, that the chemistry of the mercurial compounds employed in the treatment of syphilis materially influences their local and systemic effects. Soluble inorganic compounds like the bichlorid are the most toxic because readily dissociated; therefore, they are likely to be the most irritating when applied locally, injected intramuscularly or injected intravenously. They are most rapid in union with proteins and, therefore, most bactericidal, most corrosive and most likely to produce intravascular hemolysis and plasma precipitation. Soluble organic pseudo-or half-complex compounds, like the succinimid, flumerin and mercurosal, are less toxic because less readily dissociated, less bactericidal, less corrosive, and much less likely to produce intravascular reactions. For the same reasons they are likely to be somewhat less spirocheticidal, although the differences are more difficult to elicit because variations in curative activity in human and experimental syphilis are more difficult to express in well-defined terms and figures than is toxicity.

Toxicity of Mercurial Compounds for the Lower Animals in Relation to Toxicity for Human Beings.—We may now briefly review the results of toxicity tests employing the lower animals and the mercurial compounds commonly employed in the treatment of syphilis; these are not without some interest and practical importance in relation to the treatment of this disease.

These results are probably best expressed according to the *maximum single tolerated doses per kilo of body weight* by intravenous and intramuscular injections. In 1915 Schamberg, Raiziss and I[8] observed the following results with soluble salts, employing white rats injected intravenously:

Compound	Per Cent. Hg	By Intravenous Injection	By Intramuscular Injection
Mercury bichlorid	74	0.002	0.0065
Mercury succinimid	50	0.0018	0.014
Mercury benzoate	45	0.0024	0.0085
Mercury cacodylate	20	0.0045	0.018
Mercury salicylate	58	0.003	0.004
Mercury oxycyamid	83	0.001	
Mercury sozoiodolate	32	0.0022	0.012

In a general manner, the toxicity of these compounds, varied roughly according to the mercury content. The cacodylate was borne in the largest dose, but this was because the product which we used contained only 20 instead of the theoretical 42 per cent. of

mercury. Leaving out this compound, it is observed that the maximum single tolerated dose of these soluble compounds by intravenous injection was from 0.001 to 0.003 gm. per kilo corresponding to 0.06 to 0.180 gm. or 1 to 3 grains per 60 kilograms (approximately 132 pounds). By intramuscular injection the doses ranged from 0.004 to 0.014 gm. per kilo corresponding to 0.240 to 0.840 gm. or 6 to 14 grains per 60 kilograms or 132 pounds; toxicity by intramuscular injection was, therefore, roughly four times less than by intravenous injection.

With the insoluble compounds, as calomel, the salicylate and gray oil, the maximum single tolerated doses by intramuscular injection were found to be from 0.004 to 0.006 gm. per kilo corresponding to 0.240 to 0.360 gm. or 4 to 6 grains per 60 kilograms.

Mercurophen, the sodium salt of oxy-mercuri-ortho-nitro-phenol, containing about 45 per cent. mercury, is borne by white rats by intravenous injection in dose of about 0.008 gm. per kilo, according to tests made by Schamberg, Raiziss and myself.[9] This dose corresponds to 0.480 or about 8 grains per 60 kilos or 132 pounds.

Flumerin, according to White, Hill, Moore and Young[10] is tolerated by dogs and rabbits in dose of 0.030 to 0.040 gm. per kilo; I have confirmed these results, employing white rats. This dose corresponds to 1.8 to 2.4 gms. per 60 kilos or 132 pounds.

Mercurosal is reported to be borne by guinea pigs and dogs in dose of 0.025 gm. per kilo by intravenous injection, which corresponds to 1.5 gm. per 60 kilos or 132 pounds.

It is now interesting to note how the doses of these various compounds ordinarily administered in the treatment of syphilis compare with the maximum single tolerated doses (M.T.D.) for the lower animals; these may be expressed as follows in terms of the dose per 60 kilos or 132 pounds:

Compound (Intravenously)	Estimated Maximum Tolerated Dose	Estimated Dose for Treatment	Ratio
Bichlorid of mercury	0.120 gm.	0.01 gm.(1/6 gr.)	1:12
Succinimid of mercury	0.108 gm.	0.015 gm.(1/4 gr.)	1:7
Benzoate of mercury	0.144 gm.	0.01 gm.(1/6 gr.)	1:14
Cyanid of mercury	0.060 gm.	0.005 gm.(1/12 gr.)	1:12
Mercurophen	0.480 gm.	0.02 gm.(1/3 gr.)	1:24
Flumerin	1.8 gm.	0.18–0.3 gm.(3–5 gr.)	1:6 to 1:10
Mercurosal	1.5 gm.	0.1 gm.(2 gr.)	1:15
Mercurosal (Intramus.)	3.0 gm.	0.05 gm.(1 gr.)	1:6
Calomel (Intramus.)	0.3 gm.	0.06 gm.(1 gr.)	1:5
Salicylate of mercury (Intramus.)	0.3 gm.	0.06 gm.(1 gr.)	1:5

It will be observed from this summary that the dose of flumerin advocated for the treatment of syphilis (0.003 to 0.005 gm. per kilo) is only 6 to 10 times less than the maximum tolerated dose; the dose of mercurophen which I employ is 24 times less, the dose of mercurosal for intravenous injection is 15 times less and the other compounds from 7 to 14 (average 12) times less than the maximum tolerated doses for the lower animals. These ratios are worthy of being kept in mind when comparing one mercurial with another in the treatment of syphilis; that is to say, when superior claims are made for a compound it is well to know how the dose employed compares with the maximum tolerated dose for the lower animals, since the effects may be due more to larger doses than to any superior spirocheticidal activity on the part of the drug.

From the standpoint of intramuscular injection, it will be observed, that according to established custom, the margin of safety is somewhat less since the doses ordinarily given are only 5 to 6 times less than the maximum tolerated doses for the lower animals.

These figures, however, do not tell the whole story of the comparative toxicity and merits of the different compounds as employed in the treatment of syphilis. For example, when given intravenously, they do not show the incidence of acute reactions of fever, vomiting, diarrhea, etc., as these effects

are not ordinarily elicited in the smaller lower animals. From this standpoint, mercurosal, flumerin, mercurophen and the succinimid are to be preferred as they do not precipitate the plasma proteins as may the bichlorid, cyanid and other salts and clinically are better borne. Likewise from the standpoint of renal irritation, marked differences are shown. In my experience the salicylate, succinimid, bichlorid and benzoate are more likely to produce nephritis of the lower animals than mercurophen, mercurosal and flumerin. From the standpoint of local irritation by the soluble salts injected intramuscularly, the bichlorid is probably most irritating and mercurosal the least; mercurophen is too painful for intramuscular injection and while flumerin is less so, it has not been employed in the treatment of syphilis by this route of administration. Of the insoluble compounds, calomel is most painful followed in order by gray oil, the salicylate and mercurosal.

Pathological Tissue Changes Produced by Mercurial Compounds in Relation to Toxic Reactions and the Treatment of Syphilis.—This naturally leads one to inquire regarding the chemopathological tissue changes of the internal organs that may be produced by the administration of mercury and especially how these vary or are influenced by different compounds and different routes of administration. It is generally well known that the kidneys are especially likely to suffer and that careful examinations of the urine are indicated during the treatment of syphilis for evidences of renal irritation, but the possible changes in other organs are not without interest and importance.

Studies of this kind are best made with the lower animals because human tissues obtained at necropsy are always likely to show the presence of lesions produced by syphilis or other diseases apt to be confused with those that may be produced by mercury. In 1915 I made a study of the kidneys of rabbits treated with intravenous and intramuscular injections of various salts and well-defined tubular and diffuse lesions were observed which bore some relation to the kind of compound injected, the percentage of mercury contained and the duration of life. A few reports are to be found in literature bearing on the nephritis produced by mercuric chlorid; excellent studies of experimentally produced nephritis have been made by Elbe,[11] Burmeister and McNally[12] and MacNider.[13] These investigators have considered the kidney lesions dependent on the action of the mercury on the vascular tissues of the kidney, to the action of the metal as such on the parenchyma during elimination or to shock associated with severe mercury enteritis and the development of acid intoxication.

In 1921 Lucke and myself[14] made a rather extensive study of the histopathological changes produced by different mercurial compounds administered to rabbits by intravenous and intramuscular injection, by inunction and by ingestion; as expected, individual animals exhibited varying degrees of tissue injury, so that a comparison of the kind and severity of the lesions produced by the different preparations of mercury administered by different routes, in multiple small doses, is possible only in a broad and general manner.

The lesions produced by the different mercurials were quite similar and may be briefly summarized for the different organs as follows:

The *brain and meninges* showed an interesting and unexpected perivascular round-cell infiltration; curiously, this change is regarded as more or less constant in syphilitic infection of these tissues, and our findings quite naturally raised the question of the significance of perivascular round-cell infiltration in the brain of syphilitic subjects who have taken mercury during life. Among the animals receiving a large amount of mercury, some conges-

tion and edema with slight perivascular round-cell infiltration were noted in the pia mater. These lesions were somewhat more marked in animals which received intramuscular injections. In view of the unexpected finding of perivascular round-cell infiltrations in the brains of some of these rabbits and their practical importance in relation to similar changes produced by syphilis in the central nervous system and especially in paresis, we continued this study[15] with monkeys in view of the report by Oliver,[16] that spontaneous meningeal and cerebral lesions of perivascular round-cell infiltrations may be found in normal stock rabbits. Preliminary to this study, however, Lucke[17] examined the brains from monkeys which had died at the Philadelphia Zoölogical Garden with the result that the brains of nine animals were found to show pathological changes of non-suppurative meningo-encephalitis, arteriosclerosis with focal necroses and gliosis, etc., but with no tissue changes exactly similar to those apparently produced by mercury. Three monkeys, in good condition, were used; the first received 37 inunctions of blue ointment in dose equivalent to 4 gm. per 60 kilos at the rate of three per week; the second received 37 intramuscular injections of mercuric chlorid in dose of 0.02 (⅓ grain) gm. per 60 kilos at the rate of three per week and the third received 14 intramuscular injections of salicylate of mercury in dose of 0.06 (1 grain) gm. per 60 kilos at the rate of one per week. The histological changes were summarized as follows: (1) The pia-arachnoid was locally thickened and cellular; this was most pronounced in the sulci and was chiefly due to fibroblastic hyperplasia. (2) The endothelium of many capillaries and of the small vessels in general was large and swollen and in a state of active proliferation. New vessel formation, as evidenced by capillary budding, was frequently observed. (3) A mild and nowhere striking infiltration of the adventitial lymph space of the smallest vessels with lymphocytes, plasma cells, and intermediate forms was present. (4) There was a moderate proliferation of the cellular as well as the fiber-forming neuroglia. The former was most distinct about proliferating capillaries, in the white matter and in the neighborhood of diseased ganglion cells; the latter was best seen in the subependymal and marginal cortical layers. (5) A relatively small number of ganglion cells were in a state of acute degeneration. (6) The distribution of the lesions mentioned was irregular, mainly local in character, and on the whole most pronounced in the basal portions of the brain.

The lesions described affected chiefly the capillaries and small vessels; they were proliferative, degenerative and mildly exudative in character; they, therefore, correspond to our conception of a reaction to an irritant. A comparison with the tissue alterations occurring in the central nervous system after intoxication with two other metallic poisons, lead and arsenic, discloses an essential similarity. The neuropathologic picture of arsenical poisoning has been described by Jacob, Weygand and Kafka;[18] these observers injected solutions of arsphenamin and neoarsphenamin into the central nervous system of monkeys. The changes of lead encephalopathy have been recently summarized by Hassin,[19] who confirms the results of similar studies by Mott[20] and Bonfiglio.[21] Hassin showed that lead and arsenic induce nervous changes that may be designated as toxic nonsuppurative encephalitis. As examples of the latter group, epidemic encephalitis and poliomyelitis may be mentioned; in these infections infiltrative and exudative phenomena characterize the picture, while proliferative alterations preponderate in the toxic group.

While we are in general accord with Hassin's statements, it seems to us, however, that the lesions of certain chronic infections (i.e., syphilis) are more

difficult to separate from the infectious type mentioned above, for in syphilis proliferative changes are as prominent as, if not more prominent than, degeneration and exudation. In dementia paralytica, which is universally regarded as due to syphilis, the histopathologic picture consists essentially in new vessel formation, marked swelling of the vascular endothelium, infiltration of the adventitial spaces and the nervous parenchyma by certain round cells probably derived from the blood or lymph stream, the occurrence of rod (Stabchen) cells, proliferation of the neuroglia of all types, and various degenerative changes of the ganglion cells and other structures. The lesions of general paralysis are most commonly confined to the frontocentral parts of the cerebral hemispheres, but cases occur in which other parts of the brain are more involved.

Now these changes of general paralysis are, in their essential nature, similar to those we have just described as resulting from experimental mercurial administration. In point of severity, however, the lesions due to mercury are far less intense.

While there can be no doubt that syphilis is the essential cause of general paralysis, it is still an open question whether other factors, among them mercury, arsenic, or other metals, may not play a subordinate rôle in the establishment of this entity. It must be distinctly understood, however, that no evidence has as yet been adduced that any of the clinical manifestations of general paralysis are due to mercury. We do not wish to be understood as putting forth the claim that mercury is responsible for the principal lesions of general paralysis, but, in view of our experimental results in monkeys, we feel justified in asserting that this metal and possibly other organic poisons may produce structural alterations of sufficient degree to influence the histopathologic picture. In this connection it is well to bear in mind that the salts of mercury (as well as those of arsenic and lead) produce tissue changes in many organs (kidneys, liver, gastro-intestinal tract, etc.), and that the mercurials in particular call forth distinct alterations in the blood-capillary system. It would be remarkable if the nervous tissue should be exempted.

As a general rule, the *heart* showed no changes although in those animals receiving the maximum amounts of mercury there were evidences of slight focal necrosis of the muscle cells with intermuscular round-cell infiltration.

Changes in the *lungs*, when found at all, mainly consisted of congestion and slight edema.

The changes found in the *spleen* consisted mainly of hyperemia and hemosiderosis, apparently due to increased blood destruction.

Changes in the *liver* were usually inconspicuous and when noted at all comprised congestion, filling of the sinuses with partially hemolyzed erythrocytes and hemosiderin staining of neighboring hepatic cells. In some animals there were evidences of slight cloudy swelling of the hepatic cells and slight perilobular fibrosis.

The *kidneys* have shown the most conspicuous changes analogous to those previously described by me and every one showed some degree of tissue injury, the severity varying considerably according to the amount of mercury administered. The most constant lesions were found in the tubules characterized by cloudy swelling, fatty degeneration, frank necrosis, calcareous infiltration and the presence of large numbers of casts. The convoluted tubules suffered most; the limbs and loops of Henle were sometimes involved while the collecting tubules generally escaped. The glomeruli were sometimes hyperemic and among animals receiving the larger amounts of mercury, granular exudates occurred in the capsular spaces. Among the animals

surviving for the longer periods of time, there were evidences of repair of the tubular cells accompanied by interstitial changes of lymphocytic infiltration and fibrosis.

As far as we could ascertain, with hematoxylin and eosin-stained sections, the *suprarenal glands* showed no changes, although we reported reservedly on the histology of these tissues in this series of experiments.

Relation of the Kind of Compound of Mercury and Route of Administration to Tissue Injury.—In-so-far as the kind of compound in relation to tissue injuries is concerned, it may be stated that the lesions were essentially similar and bore a rough relation to the amounts of mercury administered in the various compounds. This is to be expected, as the mercury of the compounds employed is readily dissociated and I believe the tissue injuries are due primarily to ionized mercury regardless of the kind of compound from which it is derived. Therefore, compounds of the half- and full-complex type are least likely to produce tissue injury because they may be eliminated unchanged before dissociation or ionization can occur; soluble inorganic salts like the bichlorid, on the other hand, are apt to produce severe changes because the mercury is so quickly dissociated. When compounds of mercury are injected intravenously, however, we may produce acute changes in the blood due to variations in hydrogen ion and other physical phenomena produced by the compound in addition to those produced by the dissociated mercury.

The route of administration bore a more definite relation to the pathological tissue changes. Apparently ingestion produces the mildest changes, but the liver may be particularly involved. Intravenous injections of the soluble salts produced the severest changes, followed in order by intramuscular injections of the soluble salts, intramuscular injections of the insoluble salts and inunctions. Doubtless the degree of solubility and rapidity of absorption were the determining factors.

Of special interest in this connection is the question of absorption of calomel applied by inunction. Schamberg, Raiziss and I have found that absorption occurred when calomel was applied to the skin of rabbits, but Cole and his associates found that calomel inunctions produced much less salivation than inunctions of blue ointment, and on this basis expressed the opinion that absorption was much slower and less certain. But I am convinced of the absorption and therapeutic effects of calomel inunctions. The ordinary blue ointment is 50 per cent. of mercury; since calomel contains less mercury, it should be used in 85 per cent. ointment. Recently Cole, Hutton and Sollman[22] have advocated a clean inunction treatment of syphilis consisting in the usual daily inunctions of 4 gm. of blue ointment (50 per cent. mercury) rubbed in for thirty minutes, followed by washing the skin with benzene. They claim that the results are just as good as when the ointment is left on the skin over-night as is the usual custom. But I have found in experiments with rabbits that washing off the excess with benzene greatly reduces the degree of absorption. For example, rabbits of 1800 to 2000 gms. rubbed daily for 30 minutes with 0.5 gm. of blue ointment usually lose rapidly in weight and die with nephritis after 6 to 10 doses; rabbits rubbed in exactly the same manner, for the same length of time, but washed off with benzene and placed in a separate room, stand 8 to 12 doses. Rabbits rubbed for 30 minutes with 0.5 gm. of 85 per cent. calomel ointment usually lose rapidly in weight and die with acute nephritis after 3 to 5 doses; rabbits treated in exactly the same manner but washed with benzene and placed in a separate room, stand 8 to 12 doses. Experiments of this kind have convinced me, therefore, of two things, namely, (1) that calomel applied

by inunction is absorbed and (2) that absorption of both calomel and blue ointment is greater (probably by volatization and inspiration) if the excess is allowed to remain on the skin.

Local Tissue Changes Produced by Intramuscular Injections of Mercury. All compounds of mercury injected intramuscularly produce varying degrees of local tissue reaction. By injecting small doses into the thigh muscles of the rabbit, guinea-pig or rat, in exactly the same area, and carefully excising the tissues five days later, it was possible by means of serial sections to study the kind and degree of changes produced by different compounds injected in water and oils. I have made a study of this kind with mercuric chlorid,

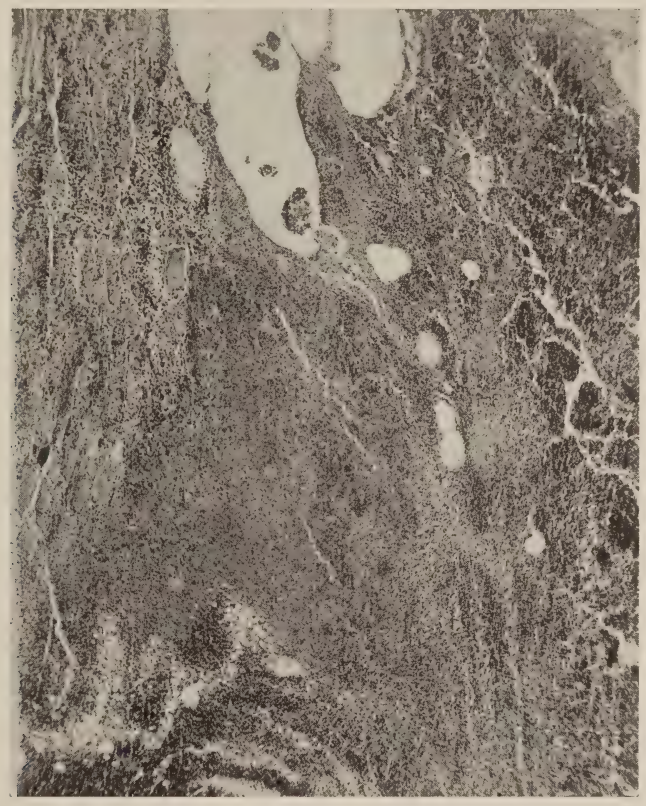

FIG. 25.—LOCAL REACTION PRODUCED BY INTRAMUSCULAR INJECTION OF SALICYLATE OF MERCURY.

Injection of 0.1 gm. in olive oil (4 days).

mercurophen, mercurosal, calomel, salicylate and gray oil with rather interesting and practical results which are here briefly summarized.

In the first place the essential tissue changes are the same for all compounds and consist of varying degrees of muscle necrosis with leukocytic infiltration, edema, congestion and fibrosis. All of the compounds studied produced reactions, the severity varying, of course, with the amounts injected (0.002—0.1 gm.). The degree of muscle necrosis, however, was less than produced by the organic arsenicals. The infiltrations were composed mainly of polymorphonuclear leukocytes followed by lymphocytes, plasma cells and fibroblasts (Fig. 25).

Soluble compounds usually produced a somewhat sharper reaction than insoluble compounds, that is, there was more necrosis of muscle, denser leukocytic infiltration, more congestion and hemorrhage and more edema. I presume that these changes were due primarily to the degree and rapidity of dissociation of mercury, since compounds of the inorganic class, like the bichlorid, which dissociate rapidly, produced more reaction than organic compounds like the salicylate and flumerin which dissociate more slowly and, indeed, may be absorbed and eliminated in part without any dissociation at all.

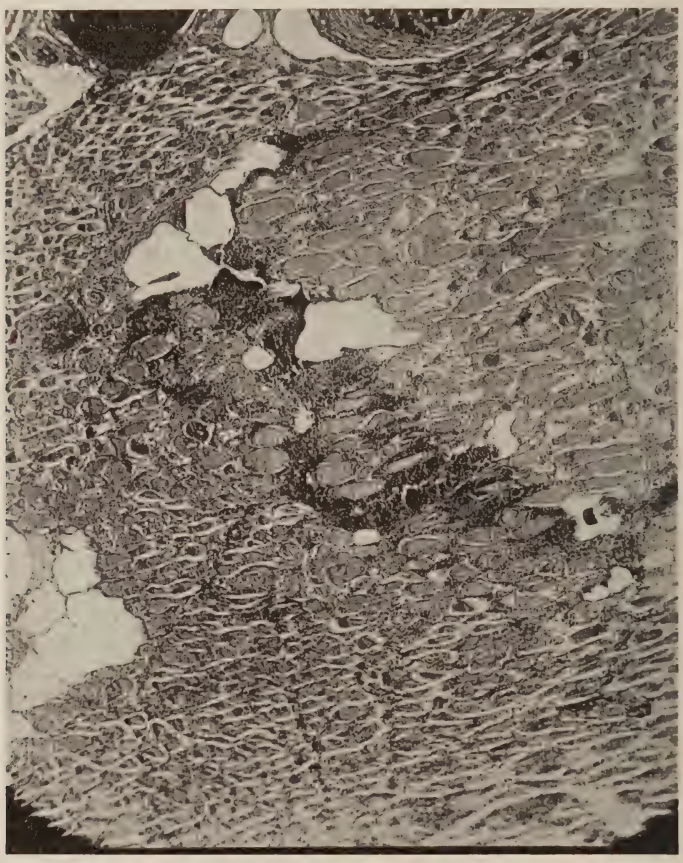

FIG. 26.—LOCAL REACTION PRODUCED BY INTRAMUSCULAR INJECTION OF CALOMEL IN OIL.

Injection of 0.002 gm. in olive oil (5 days).

Insoluble compounds produced less reaction than soluble compounds, but these varied somewhat in this relation; suspensions of calomel for example, produced more reaction than suspensions of the salicylate or gray oil, but I believe that these differences were due primarily to variations in the rapidity of dissociation of the mercury ions.

The menstrum employed modified the local reaction to a considerable extent. For example, bichlorid in water produced a sharper reaction than a similar dose in almond oil; suspensions of the salicylate in water produced sharper reactions than suspensions in oil, etc. The lesions produced by

suspensions in almond or olive oils were actually somewhat larger, but this was due to the fact that they were less circumscribed, that is, the leukocytic and serous infiltrations were more diffuse than produced by water solutions or suspensions. Suspensions in almond or olive oils were more diffuse than suspensions in paraffin oil; the latter were more circumscribed with sharper encapsulation.

It was apparent, therefore, from these histological studies, that the *degree* of local intramuscular reaction produced by injections of mercury compounds varied according to solubility, dose in terms of mercury, rapidity of dissocia-

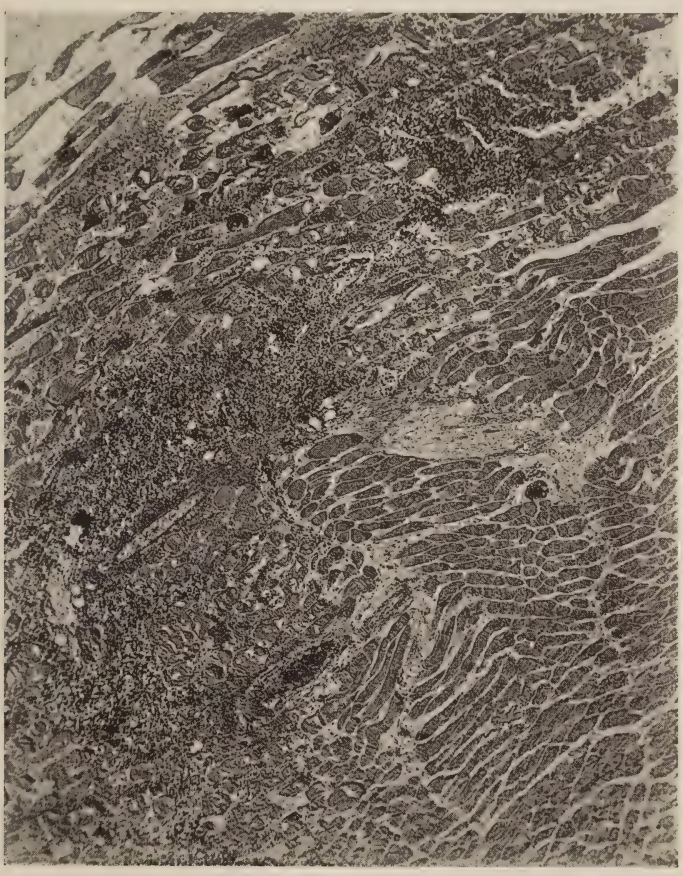

FIG. 27.—INFLUENCE OF POTASSIUM IODID UPON THE LOCAL REACTION PRODUCED BY THE INTRAMUSCULAR INJECTION OF CALOMEL.

Injection of 0.002 gm. of calomel in olive oil following the oral administration of potassium iodid for five days.

tion of mercury ions and the menstrum employed. Soluble inorganic salts of rapid ionization, like mercuric chlorid, administered dissolved in water, produced the most severe but sharply localized lesions; when administered in almond oil the reactions were less severe in the sense that they were more diffuse. Soluble compounds of slower ionization, as flumerin and mercurosal, produced somewhat less severe changes. Insoluble compounds suspended in vegetable oils varied, more reaction being produced by calomel and the metal itself (gray oil) than by the salicylate. Solutions or suspensions in water,

therefore, are apt to be more painful than suspensions in oil and suspensions in sterile almond or olive oils are to be recommended from this standpoint as preferable to the former.

In additional experiments, the drugs were injected intramuscularly to animals previously receiving solutions of sodium or potassium iodid by stomach tube for five or more days. This appeared to increase the degree of leukocytic infiltrations for all compounds as shown in (Figs. 26 and 27) as examples of the series. I do not know whether or not this possesses clinical significance, as I have not been able to satisfy myself that intramuscular injections of the salicylate, calomel or gray oil produced any more severe reactions among patients taking 10 to 30 grains of sodium or potassium iodid per day than among patients who were not taking the iodids, but these experimental studies indicated that, in a general way, the iodids tended to increase the degree of leukocytic reaction around the deposited drugs in the muscles of the lower animals.

Etiology and Mechanism of Toxicological Reactions Produced by the Administration of Mercurial Compounds. *Etiological Classification of the Toxicological Effects of Mercury Compounds.*—Upon first thought it would appear a relatively simple matter to explain the mechanism of the poisonous action of mercury upon the proposition that the metal united with cells and brought about their dysfunction or destruction. But the subject is not quite so simple and several additional factors require consideration. In the first place we must distinguish between those effects produced by the metal itself upon the tissues and the secondary effects following these; for example, McNider[23] believes that the kidney lesions in acute mercuric chlorid poisoning are not due to the action of mercury as such upon the blood-vessels and epithelium of the kidneys, but to the effects of acid intoxication resulting from the primary corrosive gastro-enteritis produced when the drug is swallowed. Furthermore, I believe that a distinction should be made between the effects of compounds of mercury and mercury itself and especially when injected intravenously.

These various factors may be classified, somewhat as follows:

Primary factors	(1) Corrosive local effects.
	(2) Degenerative effects of the compounds or of mercury itself upon the internal organs.
	(3) Effects of mercury upon the nervous system.
	(4) Hemolysis.
	(5) Precipitation of plasma proteins.
	(6) Acid intoxication.
Predisposing factors	(1) Hypersensitiveness to mercury.
	(2) Bacterial or spirochetic gingivitis; focal infection.
	(3) Gastro-intestinal, renal, hepatic and cutaneous disorders.
	(4) Syphilis (Jarisch-Herxheimer reactions).

Corrosive Local Effects of Mercury.—These refer to the local irritations produced when mercury is administered by ingestion, inhalation, inunction and by injection into the muscles, and they vary according to the compounds employed. By ingestion the inorganic compounds as a class are more irritating than the organic compounds and especially mercuric chlorid. The lesions are largely those of congestion, exudation and necrosis. In 1906 Schmiedeberg[24] expressed the opinion that the intestinal lesions were due to the destructive action of the metal during its elimination and Burmeister and McNally[25] later expressed a similar view. Mercury itself is not very irritating unless it becomes ionized, as shown by the old custom of administering very large doses for cathartic effects without injurious results, but I

have personally no doubt that quickly dissociated or ionized compounds produce direct corrosive effects before absorption, as shown not only by the rapidity with which gastro-enteritis follows the ingestion of large doses of mercuric chlorid, but also by the local tissue reactions produced when mercurial compounds are injected intramuscularly. Readily dissociated compounds, like the bichlorid and protiodid, are more apt to produce local irritation when administered orally, according to Fournier, than other compounds commonly employed in the treatment of syphilis; mercury with chalk on the other hand, is less corrosive and for this reason was the favorite of Jonathan Hutchinson. By inunction little or no differences are discernible but by inhalation, corrosive effects may be produced, more, however, by other chemical substances than by the metal itself, as shown by the disasterous effects of the old, obsolete and harbarous method of the treatment of syphilis by "perfumes."

Degenerative Effects of Mercury.—But are the lesions described above as occurring in the kidneys, liver, brain, heart muscle, etc., due to deposits of mercury in the cells, or, are they due to such secondary changes as disturbances in the acid-base equilibrium of the blood? In view of the fact that chemical analyses have regularly shown the presence of mercury in such organs, I believe that the lesions are due in most part to the necrosing or corrosive effects of mercury. Unfortunately, no thoroughly acceptable method has yet been evolved for demonstrating mercury in the cells by histological methods although Weidman[26] has worked out a method for demonstrating metallic mercury in the tissues as found by Mastin and myself in the tissues of an aortic aneurysm. I believe that ionized mercury is capable of union with cells and particularly of the kidney and intestinal mucosa and capable of producing degenerative lesions of cloudy swelling, liquefaction and coagulation necrosis followed by calcareous infiltration.

Mechanism of the Union and Action of Mercury upon Cells.—This raises the question of *how* mercury unites with cells and *how* it produces dysfunction and destruction. Unfortunately we possess no accurate information upon these subjects, but since the administration of sulphur compounds, as sodium thiosulphate, is said to reduce the toxicity of mercury, I surmise that mercury may enter into the protoplasm of cells by means of union with the sulphhydril group, as recently maintained by Voegtlin and his associates in the case of arsenic. Furthermore, it would appear as well established that larger doses of mercury are withstood and especially by inunction, when sulphur waters are freely taken internally as mentioned by Fournier in reference to the experience of Doyen at Uriage and as practiced at present at Aachen in Europe and at the Hot Springs of Arkansas in this country. How mercury interferes with the function of the molecule after this invasion has occurred is unknown; it may be by interference with sulphur metabolism, by coagulation of the proteins or possibly by interference with internal respiration by alternate oxidation and reduction. These are problems urgently requiring elucidation by pharmacologists.

Effects of Mercury upon the Sympathetic Nervous System.—Mercury undoubtedly may influence the nervous system and especially certain portions of the anatomic elements. Lesions of the peripheral nerves in the form of neuritis do not occur as frequently as in the case of arsenical and lead intoxications although tremors, irritability and rarely paralyses, may occur in chronic mercurialism. But in addition, mercury may influence the vasomotor system in a manner analogous to the action of arsenic but to a lesser degree, resulting in disturbances of blood-pressure and local lesions of congestion and edema, as seen in the changes of the tongue and buccal mucosa in

stomatitis, the skin lesions, etc. Furthermore, it would appear that the salivation of mercury without evidences of stomatitis may be due to a stimulation of the symphathetic nerves of the salivary glands, which explains in part the variations met with among human subjects in susceptibility to the salivating effects of this drug; furthermore, the increased output of urine sometimes associated with glycosuria, may be due in part to sympathetic nerve stimulation in conjunction with vasomotor changes.

Hemolysis and Precipitation by Mercurial Compounds Injected Intravenously.—Of further interest is the subject of the effect of mercury and mercury compounds upon the erythrocytes and plasma proteins. As discussed in Chapter XLV we have already shown that such intravascular reactions may be of considerable practical importance in relation to acute toxic reactions following the intravenous administration of arsphenamin and especially acid solutions.

Mercury itself in colloidal suspension does not hemolyse or agglutinate washed corpuscles or coagulate the plasma; neither do colloidal suspensions of calomel, although suspensions of the salicylate are actively hemolytic. But solutions of all of the soluble salts produce hemolysis and most of them precipitate plasma constituents. The degree of hemolysis, however, varies greatly with different compounds.

When tested in the presence of serum, however, hemolytic activity is greatly reduced, which of course reduces the significance to be placed upon the rôle of hemolysis in the production of acute reactions following the intravenous injection of mercurial compounds. Doubtless, however, a portion of the acute reaction following the intravenous injection of such a large dose of mercurochrome as 0.005 gm. per kilo in the treatment of local and general bacterial infections, is due to intravascular hemolysis.

Agglutination is not ordinarily observed; occasionally mercurophen may show slight agglutination in one or two dilutions higher than the hemolytic concentrations, but otherwise all of the soluble and insoluble salts of mercury fail to agglutinate even washed corpuscles which is in sharp contract with the high agglutinative properties of arsphenamin.

Practically all of the soluble mercurials will produce precipitates when added to serum or plasma in test-tubes. Mercuric chlorid is especially active in this particular, but the succinimid, mercurophen, flumerin, mercurochrome, and mercurosal do not, or, but to a minor degree, when very concentrated solutions are employed.

This is a subject, therefore, of some practical importance in relation to the choice of a compound for intravenous injection in the treatment of syphilis, because injections of such compounds which do not produce hemolysis or plasma precipitation as succinimid, mercurophen, flumerin, and mercurosal would appear to be indicated in preference to bichlorid, cyanid, benzoate, cacodylate and other soluble mercurials in order to avoid embolic reactions and infarctions. Of interest in this connection are the views of Heineke[27] and Kaufmann,[28] who believed that mercury in the blood had a thromboplastic action and that the damage to the kidneys depended upon the formation of thrombi with the production of infarcts.

Colloidal suspensions of mercury itself are likewise free of hemolytic and coagulating effects upon corpuscles and plasma and, contrary to what may be expected, do not tend to produce pulmonary embolism; this is especially true of the metal, which being labile, is able to slip through the capillaries of the lungs when the particles are not much larger than erythrocytes. Although the saliculate of mercury is not administered by intravenous injection, it is interesting to note in passing that it is quite hemolytic. The intravenous injection of coagulating mercurials, as the bichlorid, may excite a reaction of the character of the non specific protein reaction, possessing some therapeutic activity as claimed for mercurochrome in the treatment of local and general bacterial infections, but the doses required are very large in comparison with the maximum tolerated doses and the practice therefore is not devoid of considerable risk.

Just how hemolysis and plasma precipitation are produced is not definitely known, but I surmise that the effects are due to ionization of the mercury. The degree of activity of different compounds does not depend upon the content of mercury but salts of the inorganic acids are more active than those of organic acids. Therefore, compounds that are readily ionized are most active in these particulars and especially the soluble inorganic salts; half- and pseudo-complex compounds are much less active and full-complex compounds may be absorbed and eliminated before dissociation and these changes are produced at all.

Acid Intoxication in Relation to the Toxicology of Mercury.—As previously stated, MacNider has found that injury to the kidneys of dogs poisoned by mercuric chlorid has been constantly associated with the development of acid intoxication and that the delayed injury of these organs is due to this condition rather than to the action of mercury as such during its elimination. MacNider[29] has also found that in dogs with naturally acquired chronic-glomerulonephropathies, a state of acid intoxication is readily induced by chloroform and that this condition may be prevented by the intravenous injection of a solution of sodium carbonate equimolecular with a 0.9 per cent. solution of sodium chlorid. On the basis of these observations, Weiss[30] has developed an apparently successful treatment for acute bichlorid poisoning consisting of the internal and intravenous injection of sodium carbonate to which further reference will be made in this chapter in considering the treatment of mercurial poisoning.

Undoubtedly, this disturbance of the acid-base equilibrium is a very important facter in acute mercurial poisoning whether the drug is taken internally, is injected or is absorbed from the vaginal mucosa. Whether changes in the alkali reserve occur in milder forms of mercurialism, as in stomatitis and ptyalism, developing during the treatment of syphilis cannot be stated, but it is always my custom to administer sodium carbonate or bicarbonate internally in the treatment of such on the basis that minor disturbances of this kind may be present.

Hypersensitiveness to Mercury in Relation to Toxic Reactions.—As is now well known some individuals possess a high degree of natural hypersensitiveness to mercury and the merest contact with solutions of mercury may elicit in them severe reactions frequently expressed in the form of scarlatiniform eruptions. Others are rendered acutely ill, with vomiting, colic and diarrhea when taking internally even a fraction of the usual amounts of mercury, and it is believed that some of the fatal mercurial poisonings have occurred among hypersensitive individuals. In two cases that have come under my observation, skin reactions were elicited by the application of 1:100 solutions of mercuric chlorid to scratches on the arm.

Not infrequently one encounters syphilitic individuals who cannot tolerate mercury in any form by reason of nutritional disturbances, loss of weight, stomatitis, diarrhea, etc. These effects are probably due to lesser degrees of hypersensitiveness but when mercury is administered in any form it is well to observe closely for such symptoms in order to ascertain the individual tolerance. When administered intravenously not more than one tenth of the usual dose should be injected at first, in order to avoid or reduce what may prove to be serious and even grave reactions if the individual happens to be hypersensitive.

Whether or not hypersensitiveness is actually acquired during the prolonged administration of mercury in the treatment of syphilis cannot be stated. Not infrequently this condition appears to develop, but whether it is a true allergic hypersensitiveness or an intolerance due to accumulative toxic effects cannot always be determined or stated.

Gingivitis in Relation to Mercurial Ptyalism and Stomatitis.—Three of the most common toxicological effects of mercury are ptyalism, gingivitis and stomatitis. These effects are probably due to the excretion of the drug by the salivary glands and the buccal mucosa; stomatitis is especially likely to develop from inunctions, but most danger is from massive doses of the insoluble salts administered by intramuscular injection. It is a well established clinical fact, however, that pre-existing bacterial or spirochetic gingivitis greatly increases the incidence of ptyalism, siderosis (blue line), gingivitis and even of stomatitis following the administration of mercury. Just how these infections predispose is not definitely known, but probably by lowering resistance, interfering with elimination and by increasing the amounts of sulphur present with the production of mercury sulphid which is insoluble and irritating. Therefore, it is advisable and indeed imperative to inspect the gums of every patient about to start mercury medication and frequent inspections are required during its administration for siderosis, halitosis (foul breath), gingivitis and stomatitis, to which further reference will be made later in this chapter.

Whether or not focal infections at the apices of the teeth, in the accessory sinuses, the tonsils or elsewhere are important in relation to mercurial dermatitis and other toxic reactions, as believed by Stokes and others in relation to arsphenamin dermatitis, cannot be stated; but I believe that apical abscesses especially predispose the patient to mercurial gingivitis which in turn may readily enough favor the dissemination of bacteria from such foci so that I always insist upon a roentgenologic examination of the jaws for such foci as part of the preliminary hygiene of the mouth in preparation for the administration of mercury or bismuth.

Gastro-intestinal, Hepatic, Renal and Cutaneous Disorders in Relation to Mercurial Intoxication.—When mercury is administered by ingestion, it is particularly important that the gastro-intestinal tract shall be in a healthy state. Curiously while inunctions are especially apt to produce stomatitis, lower gastro-intestinal lesions and symptoms are much less likely to occur; Fournier states that inunctions rarely cause gastro-intestinal disturbances. It is a well-known clinical fact that the oral route of administration is unfavorable for those suffering with chronic gastro-intestinal, hepatic and gall-tract disease, not only because of the added corrosive effects of mercury, but because these organs are actively concerned in its elimination. Such individuals, therefore, are seldom fit subjects for mercury by any form of administration and least by ingestion; sooner or later their gastro-intestinal or hepatic symptoms are intensified with colic, diarrhea, a sub-icteric tinge of the skin and mucous membranes, loss of weight, etc.

Since mercury is so largely eliminated by the kidneys, there is risk in its administration if nephritis is present, not only because of the danger of interference of elimination with accumulative toxic effects, but because the degree of renal injury may be increased with the production of this vicious cycle of events. However, in syphilitic nephritis, the urinary changes sometimes improve under mercurial treatment, but it is a wiser plan to administer bismuth which is less renotoxic, or, better still, arsphenamin or neoarsphenamin, if treatment must be given. These cases represent some of the most difficult problems in the treatment of syphilis, but the order of drugs from the standpoint of least renal irritation are as follows: (*a*) the iodids; (*b*) arsphenamin and sulpharsphenamin; (*c*) neoarsphenamin; (*d*) bismuth and (*e*) mercury.

Skin lesions are of most importance in relation to inunctions of mercury. When the skin is very dry the epithelial cells are dried up, lose their resistance,

with the result that the sweat and sebaceous ducts are open and dry, as described by McConnell[31] in relation to the industrial dermatoses, permitting the greater absorption of mercury with more rapidly developing stomatitis and gingivitis as shown by Cole, Hutton and Sollmann.[32] Cole and Littman[33] have also recorded a case of severe mercurialism resulting from the rapid absorption of calomel ointment through superficial ulcero-crustaceous lesions of the skin of the entire abdomen.

Relation of Syphilis to Mercurial Intoxication and the Jarisch-Herxheimer Reaction.—Does syphilis alter susceptibility to mercury? I believe it undoubtedly increases tolerance for this drug in a most unmistakable manner in the majority of cases. Of course, susceptibility to mercury varies considerably among human beings and to a lesser extent among the lower animals, but the average tolerance of the syphilitic is higher than the average tolerance of the non-syphilitic healthy individual. One is frequently surprised with the large doses borne without any discernible ill effects by syphilitic individuals and especially in acute syphilis or very active tertiary syphilis as paresis. Kolle[34] states that syphilitic rabbits tolerate more mercury than normal controls per kilo of body weight, and, while I have not experimented along these lines, I am prepared to believe that it may be possible to define this increased tolerance of syphilized animals experimentally. How and why there should be this increased tolerance in syphilis cannot be defined; it would appear, however, that *Spirochæta pallida* and its products detoxify mercury in some unknown manner.

The Jarisch-Herxheimer reaction is mentioned in connection with this subject of the etiology of toxic reactions by mercury for the sake of completeness and not because I believe it to be a direct toxic effect of the drug. As stated in Chapter LVIII, Jarisch, Kraus and Herxheimer described exacerbations of the cutaneous and mucous membrane lesions of the secondary stage of syphilis following the administration of mercury. We now know that a similar exacerbation may occur in the chancre and more particularly in the chronic lesions of the central nervous system, but less frequently than observed after the administration of arsphenamin and its substitutes. The commonly accepted theory is that mercury releases inflammatory substances by destroying spirochetes which increases the degree of vascular changes, cellular and serous exudation, but I have discussed this subject with detail in Chapter LVIII.

Fatalities Due to Mercury in the Treatment of Syphilis.—It is impossible to even estimate the fatal intoxications from mercury since introduced into Europe for the treatment of syphilis about 425 years ago. Doubtless, however, there was a high mortality in the first two hundred years, if one may judge from the accounts of the barbarous and reckless fashion with which the drug was administered and especially be inunction and fumigation. Gaspard Torella in 1497 wrote: "Avoid, like the plague, these murderous ointments of charlatons, who have already so many victims." Rabelais congratulated Gargantua on having relieved the sufferings "of the poor syphilitics, anointed to the point at which their teeth started like the keys of an organ when played on." Ulrich de Hutten relates a case in which three patients were suffocated to death in one of the fumigating "stoves."[35]

In 1913 Wolffstein[36] reviewed some 108 fatalities from mercury in the treatment of syphilis recorded in the literature since 1883. Of these deaths only one was the result of oral medication due to five pills of one grain (0.06 gm.) each of mercury; evidently this patient was hypersensitive. There was one death from inhalation and nineteen from inunctions. Seven resulted from injections of soluble salts and seventy-eight from insoluble salts, thirty-

one of the latter being due to gray oil (*oleum cinereum*). Ehrlich and Hata stated about twelve years ago that up to that time at least eighty deaths had been caused by injections of gray oil. Of course, these figures do not include all of the fatalities from mercury administered in the treatment of syphilis; doubtless an equal or even larger number have never been recorded at all although the mortality at present per incidence of cases is undoubtedly smaller than reported for arsphenamin since its introduction for the treatment of syphilis fourteen years ago.

Prophylaxis of Toxic Reactions from Mercury in the Treatment of Syphilis.—This naturally brings us to a more complete discussion of the prophylaxis of toxic reactions when mercury is administered for the treatment of disease and especially of syphilis. Ordinarily this consists of reducing excessive smoking and an inspection of the gums and teeth with appropriate preliminary treatment to reduce the chances of ptyalism, siderosis, gingivitis and stomatitis; the correction of gross dietetic errors and constipation to reduce the chances of gastro-intestinal disturbances of diarrhea, colic, malaise and loss of appetite and finally a careful examination of the urine for albumin and casts, as indicative of the condition of the kidneys, especially if previous treatment with mercury, bismuth and arsphenamin or its substitutes had been given. In exceptional cases renal functional tests may be required, but liver functional tests are seldom required as is sometimes the case when the organic arsenicals are being administered.

But in addition to these primary considerations other factors are worthy of consideration and especially the kind of compound employed and the route of administration; both bear an important relation to the prevention of various toxic reactions by mercury.

Prevention of Ptyalism, Gingivitis and Stomatitis.—Of most importance is the condition of the mouth. If this is found healthy, treatment may be started at once, but otherwise the patient should be referred to a dentist for the extraction of stumps, the filling of cavities, removal of tartar and treatment of gingivitis if present; excessive smoking should be forbidden. During treatment with mercury the mouth should be inspected at frequent intervals and the patient requested to brush the teeth and gums night and morning with a good dentrifice. Patients should always be informed that they are taking mercury and is well to educate them with regard to the buccal accidents so that they may discontinue treatment upon the first appearance of ptyalism, halitosis, bad taste or soreness of the gums. Mercurial treatment should be always suspended on the least sign of buccal irritation and not resumed until all symptoms have disappeared. With these precautions salivation is quite uncommon and indeed, rare, when the patient cooperates with the physician. In hypersensitive individuals, however, salivation and even stomatitis may develop without warning and especially when intramuscular injections of large doses of insoluble salts (gray oil) especially are given.

Fournier states that in therapeutic doses protiodid of mercury causes more salivation than mercuric chlorid; I believe this is true, whereas, Hutchinson's old favorite mercury with chalk is well borne. Inunction is more likely to produce buccal accidents than ingestion, characterized by sudden onset, intensity and diffuseness of the stomatitis. Intramuscular injections of the insoluble salts, and especially of gray oil, are particularly dangerous because of slow absorption and accumulative action. Therefore, when it is necessary to give mercury to cases with "bad mouths," great case is required in the choice of compound and route of administration. My favorite start is with 1 grain of mercury and chalk three times a day and, if well borne, inunctions

of calomel or blue mass are given later. Injections are not given at all unless absolutely required in which case preference is given small doses of salicylate in almond oil because this salt is more soluble in the body juices and less likely to give an "explosive" stomatitis than calomel or gray oil.

Prevention of Gastro-intestinal and Nutritive Disturbances.—Some patients cannot take mercury in any form on account of gastric and intestinal disturbances as loss of appetite, pain and diarrhea. Administration by mouth may be particularly disagreeable in these respects and for this reason opium is sometimes incorporated. Mercury with chalk, however, is sometimes tolerated when protiodid and bichlorid cannot be given. Inunctions are much less likely to produce these symptoms; likewise intramuscular and intravenous injections. Constipation should always be corrected; also errors in diet and excessive drinking of alcoholic beverages.

Of great importance in this connection, as emphasized by both Fournier and Hutchinson, is intermittent treatment in order to give the organs of absorption and elimination a rest and avoid over accumulation of mercury.

Sometimes patients are better able to withstand mercury when it is administered by inunction along with hot baths and the drinking of sulphur waters. Fournier was confident that this was the case with patients treated by Doyen at Uriage and it doubtless constitutes an important part of the success of the inunction treatment of syphilis at Aachen and the Hot Springs of Arkansas at the present time. It is highly probably that the sulphur is absorbed in sufficient amounts to detoxify mercury or in some manner minimize its injurious cellular effects.

Prevention of Renal and Hepatic Disturbances.—The condition of the kidneys is always a matter of paramount importance during the administration of mercury and upon the first appearance of albumin, casts and erythrocytes in the urine, not otherwise accounted for, administration should cease until the evidences of renal irritation have disappeared. Ingestion is least likely to produce nephritis, but my impression is that this route is generally contraindicated in the presence of hepatic disorders probably because of direct absorption in the portal circulation. As a general rule, mercury should not be given at all in the presence of evidences of nephritis or recent recovery therefrom, but if it must be administered preference should be given small intramuscular doses of the salicylate in almond oil every ten days, because a portion of this is absorbed and eliminated before dissociation; or, intravenous injections of flumerin may be given, which is less likely to produce renal irritation than the bichlorid, succinimid or cyanid of mercury, probably for the same reason.

Prevention of Local Reactions.—When intravenous injections are given phlebitis and sclerosis of the veins and thrombosis may be avoided by using dilute solutions and injecting with a small (No. 22) needle. It is also well to use three or four veins or locations in rotation, which is not a difficult matter with most patients, even on the same arm.

Pain and tenderness from injections can be controlled to some extent by giving the injections epifascially (intermuscularly) rather than intramuscularly, as described in Chapter LX. By incorporating an anesthetic, as 0.1 c.c. of 2-per cent. butyn in each injection, the immediate pain is prevented. But the anesthetic does not control the late pain and tenderness sometimes requiring patients "to eat off the mantel" for a few days. These, however, are frequently caused by injecting too superficially or too near the sciatic nerve. Injections of calomel are usually more painful than injections of the salicylate and gray oil; the salicylate in almond oil is probably the least painful. The soluble salts and particularly bichlorid frequently produce

considerable pain although much less when given in almond oil than when dissolved in water or saline solution.

Prevention of Acute Toxic Reactions from Intravenous Injections.—These cannot be always avoided and especially if large doses are given but the incidence and severity are greatly reduced by two factors, namely, dilution and slow injection. Flumerin for example, is generally given in a 2 per cent. solution, 15 c.c. carrying 3 grains for an average adult or 0.003 gm. per kilo; but I believe it is better to use a 1-per cent. solution and inject 30 c.c. very slowly with a No. 22 needle. Mercurosal, mercurophen and the succinimid may be injected intravenously usually without reactions in ordinary doses, but always slowly and well diluted. Colloidal calomel and colloidal mercury are also well borne, the former being widely employed in Germany in dose of ¼ gram (0.015 gm.) for an adult. Bichlorid, benzoate, cyanid and other soluble salts should not be injected because of their hemolyzing and precipitating effects.

Contraindications to the Administration of Mercury.—Hypersensitiveness constitutes the chief contra-indication to the administration of mercury in any form and by any route. Some individuals are so hypersensitive that even swabbing or gargling the throat with 1:2000 solutions of mercurophen or bichlorid may produce acute intoxication with scarlatiniform rashes; others are less hypersensitive but yet rendered perfectly miserable by even small doses regardless of the route of administration. It is well not to force mercury under such conditions and especially since we have other antisyphilitic remedies today which the older syphilologists did not possess. Fixed mercurial dermatoses also contra-indicate the administration of mercury because of exacerbations of the lesions.

Nephritis, and especially acute nephritis whether syphilitic or otherwise, is usually a contra-indication; severe gingivitis (pyorrhæa alveolaris), aphthous stomatitis and acute or chronic gastro-intestinal disorders are likewise contra-indications in most instances and especially since other remedies like neoarsphenamin are available. Pregnancy is not of itself a contra-indication.

Etiology, Symptoms and Treatment of Toxic Reactions Produced by Mercury in the Treatment of Syphilis. *Clinical Classification of the Toxic Reactions of Mercury.*—Despite the usual care and precautions in the administration of mercury in the treatment of disease, various toxic effects may be produced and especially when patients ignore advice and directions and neglect to properly coöperate with the physician. Furthermore, when the insoluble salts are administered by intramuscular injection, toxic effects may occur with great suddenness, due to delayed absorption followed by sudden absorption of excessive amounts.

The various lesions may be grouped from the clinical standpoint somewhat as follows:

Acute colloidal shock reaction	
Gastro-intestinal disorders	Salivation or ptyalism
	Siderosis and halitosis
	Gingivitis and stomatitis
	Gastritis and ileo-colitis
Renal disorders	Polyuria
	Albuminuria
	Nephritis
Cutaneous disorders	Simple erythematomacular rashes
	Pruritus
	Scarlatiniform erythema with desquamation
	Erythema multiforme
	Dermatitis exfoliativa
	Exacerbations of syphilitic rashes (Jarisch-Herxheimer reactions)

Disorders of the nervous system	Neuritis
	Neural Jarisch-Herxheimer reactions
	Neurorecurrences
Disorders of nutrition	Chloro-anemia
	Anorexia, fatigue and emaciation
	Caries of bone
Accidents of injection	Phlebitis and thrombosis
	Pain, nodosities and sloughing
	Pulmonary embolism

Acute Colloidal Shock Reaction from Intravenous Injections of Mercury.—Various soluble salts of mercury are administered by intravenous injection in the treatment of syphilis and especially the succinimid, benzoate, cyanid and more recently, flumerin, mercurophen and mercurosal; mercurochrome is sometimes administered by this route for the treatment of bacterial injections, as already described and discussed in Chapter VIII.

In the doses ordinarily given for the treatment of syphilis, acute reactions are but rarely encountered. When mercurochrome is administered in a dose of approximately 0.005 gm. per kilo in the treatment of local and general bacterial injections, an acute reaction of fever, chilliness, leukocytosis and diarrhea may develop within an hour or two which may be due to acute colloidal shock analogous to the shock following intravenous injections of colloidal preparations of the heavy metals in general; doubtless, similar reactions would follow the injection of other salts of mercury if the doses were proportionately as large as that of mercurochrome which is given in about one-half to one-third of its maximum tolerated dose, whereas in the treatment of syphilis the dose of the other compounds just mentioned, is usually one-sixth to one-twelfth or less of their maximum tolerated doses as stated on page 740.

White, Hall, Moore and Young[37] state that intravenous injections of flumerin in dose of 0.003 to 0.005 gm. per kilo is only exceptionally followed by slight nausea, but sometimes by severe retching and vomiting tending to subside spontaneously within half an hour. They suggest that these effects may be central in origin and that dosage, number of injections given, concentration of solution, and rapidity of injection apparently exert no influence in their production. Cole, Driver and Hutton[38] state that intravenous injections of mercurosal in dose of 0.2 gm. sometimes produced "weakness" for the following twenty-four hours, but no other ill effects. Stokes has employed intravenous injections of the succinimid in dose of ¼ grain (0.015 gm.) without ill effects and I have administered mercurophen intravenously in dose of ⅓ grain (0.02 gm.) during the past three years without reactions. Monges and Raybaud[38a] record a case of fatal edema of the lungs following intravenous injections of mercury cyanid.

It would appear, therefore, that when mercurials are injected intravenously in doses not larger than one-sixth of their maximum tolerated doses, that acute reactions occur in but a small percentage of cases; when the dose is one-twelfth or less than the maximum tolerated dose acute reactions are practically unknown. This is especially true of pseudo- and half-complex compounds which do not dissociate and ionize rapidly. The late effects, however, as salivation, stomatitis, colic and nephritis may follow intravenous injections.

The *treatment* of acute colloidal shock reactions is purely symptomatic and along the same lines described for the treatment of the acute physico-hemoclastic reaction sometimes produced by arsphenamin (page 674).

Mercurial Salivation (Ptyalism).—An increased flow of saliva sometimes associated with a metallic taste is one of the earliest signs of mercurialism.

This may not be accompanied by any evidences of inflammatory changes in the gums or buccal mucosa and has been ascribed to the action of mercury upon the sympathetic nerves of the salivary glands along with direct irritation of these tissues by the excretion of mercury. In some cases, however, mercury has not been found in the saliva, the effects being ascribed purely to stimulation of the vasomotor nerves with extreme dilatation of the vessels, and may occur within a matter of ten or fifteen minutes after administration.

The tendency to salivation varies greatly among different individuals probably because of variations in susceptibility to vasomotor and autonomic disturbances. No compound of mercury is entirely free of these effects although they vary greatly in this respect. Inunctions and massive intramuscular injections are especially likely to produce the disorder. Salivation always accompanies mercurial gingivitis and stomatitis; when it occurs alone, due care must be exercised of course in diagnosis if the iodids are being taken at the same time since the latter, and not the mercury, may be responsible.

Treatment for salivation alone is not required beyond stopping medication. Local mouth washes may give some relief but do not materially influence the salivary glands.

Mercurial Siderosis and Halitosis.—The "blue line" of the gums (siderosis) and foul breath (halitosis) are well-known signs of mercurialism. Both, and especially the former, may develop insiduously without evidences of sufficient salivation to attract attention. Halitosis is especially likely to accompany severe stomatitis.

The "blue line" first develops in the free margin of the gums of the lower jaw about the central and lateral incisors and is easily detected by inspection. Infected gums (pyrorrhæa alveolaris) are especially likely to develop siderosis. According to most investigators the "blue line" is due to deposits of mercury sulphid in the endothelial cells of the capillaries followed by deposits in the connective tissues of the gums. Mercury is excreted by these channels and is believed to be converted into the insoluble and irritating sulphid by hydrogen sulphid in the tissues produced by bacteria; hence, in dirty mouths and cases of bacterial gingivitis, the "blue line" is especially prone to appear because hydrogen sulphid is particularly apt to be present.

Halitosis or "bad breath" is not due merely to the presence of mercury in the saliva since the metal is odorless; it is due rather, to putrefactive changes brought about by bacteria and yeasts in the gums, caries teeth, tonsils and saliva; sometimes it is due to gastro-intestinal disorders rather than to local conditions in the mouth.

There is no *treatement* for siderosis aside from cessation of mercurial medication. It is not always necessary, however, to interrupt a short course of treatment when it appears although the dose should be reduced. There is ordinarily little danger of gingivitis and stomatitis if the gums are otherwise sound and healthy and the patient duly careful in the cleansing of the mouth and gums twice daily. It gradually disappears although the time required dpends upon the amount of mercury to be eliminated and the degree of bacterial activity in the gums. Careful hygiene of the mouth before and during mercurial medication are of great importance in the prevention of these accidents. The *treatment* of halitosis requires a careful clean up to the mouth, correction of dietary disorders and gastro-intestinal disturbances including constipation if present and the frequent use of a mouth wash of which my favorite is a mixture of a solution of potassium chlorate and *liquor antisepticus* properly diluted.

Mercurial Gingivitis and Stomatitis.—Localized or diffuse inflammation of the gums (gingivitis) usually follows the "blue line," but may develop

suddenly and without warning; local or diffuse inflammation of the buccal mucosa (stomatitis) and of the tongue (glossitis) usually follow the gum lesions, but sometimes, and especially when large amounts of mercury are suddenly absorbed, develop without warning.

These inflammatory changes are ascribed to excessive irritation of the tissues by the excessive excretion of mercury along with vasomotor changes produced by the metal; Besseren[39] has recently reviewed the literature in a thorough manner and subscribes to the view that the changes are due to local effects superimposed on more general or widespread vascular and other changes which made possible the local lesions.

As a general rule gingivitis and stomatitis follow siderosis of the gums and cheek because the deposits of mercury sulphid produce necrosis of the tissues and thereby open up avenues of bacterial infection. The gums are tender, soft, red, bleed easily and recede from the roots of the loosened teeth which become grayish black and tend to caries. The buccal mucosa becomes swollen and tender with grayish-yellow ulcers due to contact with the teeth and especially the molars of the lower jaw. The tongue becomes swollen, flabby and indented with impress of the teeth; irregular, broad and shallow ulcers appear on the edges. The fauces may become red and swollen causing dysphagia, dribbling of saliva and fetid breath.

With careful hygiene of the mouth and careful dosage, stomatitis is uncommon. Fournier stated that he did not see more than one case in fifty with the methods practiced by him. Women are more likely to be affected than men. Intravenous injections of mercurosal in doses of 0.05 to 0.1 gm. every three to five days have rarely produced salivation and stomatitis because these doses are at least fifteen times smaller than the maximum tolerated dose; flumerin, on the other hand, has produced salivation and stomatitis in about 30 to 36 per cent. of cases, because the doses (0.003 to 0.005 gm. per kilo) are so large, these amounts being about one-sixth to one-tenth of the maximum tolerated doses. I have never observed salivation or stomatitis following a series of intravenous injections of mercurophen in dose of ⅓ grain (0.02 gm.) to adults, because this amount is at least twenty times below the maximum tolerated dose.

In other words salivation, gingivitis and stomatitis bear a relation to the degree of dissociation and ionization of mercury before elimination occurs and this in turn is influenced by the kind of compounds administered and the size of the dose.

In the *treatment* of gingivitis and stomatitis mercurial medication should be stopped, smoking stopped or greatly reduced, and the gums and mouth frequently cleansed with diluted *liquor antisepticus* or with a mixture of this solution and one of potassium chlorate. As a general rule, these measures are sufficient as the lesions tend to disappear rather promptly when the mercury is stopped unless injections of the insoluble salts have been given when they may continue for an indefinite time depending upon absorption. Dennie states that intravenous injections of sodium thiosulphate are helpful and these may be tried in severe cases after the plan described on page 695 for the treatment of arsenical dermatitis. I have also employed mouth washes of 10-per cent. solutions of sodium thiosulphate, after each meal and at bed time, followed by diluted *liquor antisepticus*, apparently with some good effects. In severe cases the diet should be bland and of necessity usually liquid, because of the pain attending efforts to masticate food. The free use of alkaline waters is helpful.

Mercurial Gastro-enteritis.—When mercury is swallowed accidentally or with suicidal intent, mercuric chlorid being usually taken, a very acute and

almost immediate gastritis with vomiting may occur, due largely to local irritation of the gastric mucosa. When mercury is administered in the treatment of syphilis, and especially the protiodid and bichlorid, colic and diarrhea sometimes develop. Mercury administered by inunction, inhalation or injection, however, may produce exactly the same symptoms.

These effects may be due, therefore, to direct local irritation when mercury is administered by ingestion or to the effects of mercury during elimination by the bile and intestinal mucosa after any method of administration. The mechanism is apparently exactly similar to the production of gingivitis and stomatitis in that the mercury produces vascular changes of congestion and hemorrhage along with direct irritation of the vascular endothelium and tissues of the mucosa during elimination. Siderosis occurs as in the mouth, due to deposits of mercury sulphid from the hydrogen sulphid of the intestinal bacteria; this in turn may be followed by ulceration and especially of the mucosa of the colon, although ulcers may be produced without deposits of mercury sulphid.

Some individuals are absolutely unable to take even fractions of the usual doses of mercury by any route on account of gastro-intestinal disturbances. As a general rule, diarrhea is the first development, occurring in a few hours or after a few days following administration. Nausea, vomiting, vertigo, thirst and colic with gaseous distention may precede or accompany the diarrhea. The stools are watery, fetid and accompanied by tenesmus; later blood, pus and even shreds of intestinal mucosa may be present.

The frequency of gastro-intestinal reactions varies greatly with different individuals, the compound used and the method of administration. Oral administration is more likely to produce them than inunctions or injections; mercury with chalk is oftentimes well borne when the protiodid and bichlorid cannot be given. Intravenous injections of flumerin at two-day intervals produces about 4.3-per cent. reactions of immediate nausea and vomiting but less than 1 per cent. of diarrhea and cramps. As in gingivitis and stomatitis, these ill effects vary with the compound in relation to the rapidity of its dissociation and ionization and the amount of mercury contained.

In *treatment* the first step is to stop mercurial medication. The diet should be bland and largely of milk. Cathartics are not required because peristalsis and intestinal cramps or contractions are severe enough. Bismuth subgallate with opium should be given until relief is obtained. Alkaline drinks are helpful as one teaspoonful of potassium tartrate and one-half teaspoonful of sodium citrate in a glass of water, orangeade or lemonade.

Renal Disorders Due to Mercury.—Since mercury is largely eliminated by the kidneys it is not surprising that these may be involved early in acute and chronic mercurialism. In acute bichlorid poisoning, the nephritis with anuria and uremia constitutes the most dangerous lesions, but during the treatment of syphilis signs of renal irritation and nephritis must be looked for routinely and especially if intravenous injections are being given.

The first sign is polyuria due to the increased vascular engorgement of the organs as a result of the influence of mercury upon the vasomotor system. Glycosuria may appear as a result of sympathetic stimulation, hepatic disturbances or increased permeability of the renal cells. Polyuria and glycosuria, however, are not usually detected during the treatment of syphilis since the onset of renal irritation is so mild and gradual. As a general rule albuminuria is the first sign of renal disturbance accompanied by casts and, in severe cases, by blood and much tubular epithelium. Anuria may now develop and constitute the great and chief danger of acute mercurial poisoning.

The histological changes have been already described. Opinions vary, however, on the mechanism of production of these changes. Most investigators of this subject believe that the changes are essentially the result of direct irritation of the glomeruli and tubules by mercury, the peculiar distribution of the lesions being due to distribution of the blood supply and drainage of these organs. Others, and especially MacNider, believe that the renal changes are largely due to acid intoxication and especially in acute mercurial intoxication with severe gastro-enteritis. In my opinion, the renal changes sometimes developing in the treatment of syphilis, like albuminuria with casts and decreased phenolsulphonphthalein return, are due to vascular engorgement of the glomeruli as a result of the influence of mercury upon vasomotor nerves and to cloudy swelling and necrosis of the glomerular and tubular epithelium, especially of the collecting tubules, by mercury in excessive amounts in the blood brought to these organs for elimination. Doubtless acid intoxication may be an added factor in the production of cloudy swelling and necrosis, but only in acute poisoning, like bichlorid poisoning, with severe gastro-enteritis. Bessesen and some other investigators believe that mercury probably forms a compound with the blood, as indicated by a decreased blood count and hyperemia of bone-marrow, with a reduction of oxygen and an increase of lactic acid, which, together with the mercury, act on the sympathetic system as irritants in addition to the formation of albuminates of mercury in the cells of the kidney rendering them less active.

The frequency of renal changes during the mercurial treatment of syphilis varies greatly according to the nature of the compound administered, as influencing the rate of dissociation and ionization of the mercury, the route of administration and the content of mercury. When treated by ingestion, renal irritation is very uncommon, as shown by the experience of Fournier and Hutchinson, the two great exponents of this route of adminstration.

Inunctions produce more irritation, as likewise intramuscular injections, and especially intravenous injections of the soluble salts. It is always advisable to examine the urine for albumin and casts at least once a week or fortnightly during active mercurial medication. Flumerin is reported by White and his associates as producing transient albuminuria in about 22 per cent. of cases with decreased phenolsulphonphthalein return in about 5 per cent., when the usual doses of 3 to 5 gm. per kilo are injected intravenously at two day intervals; mercurosal, mercurophen and succinimid apparently do not produce as much irritation largely because the doses are comparatively much smaller.

The *treatment* of mercurial nephritis developing in the course of mercurial medication in syphilis does not usually require anything more than stopping the drug. As a general rule, the albuminuria gradually disappears, although this depends upon the amount requiring elimination. In acute mercurial nephritis, however, more energetic measures are required and especially in bichlorid poisoning. A large number of methods have been described. Weiss has reported unusually good results from the intravenous injection of 1000 to 1500 c.c. of Fischer's solution, prepared by dissolving 10 gm. of crystallized sodium carbonate and 15 gm. of sodium chlorid in 1000 c.c. of distilled water followed by sterilization or, of a sterilized 4-per cent. solution of sodium bicarbonate in water. In severe poisoning the infusion may be required a second or third time on successive days, the object being to correct the acid intoxication and render the urine alkaline to methyl red and to keep it so; 8 ounces of "imperial drink" are given internally six to eight times a day (1 teaspoonful of potassium bitartrate and ½ teaspoonful of sodium citrate in 8 ounces

of water, orangeade or lemonade). In addition to this alkaline treatment it may be serviceable to administer daily intravenous injections of sodium thiosulphate in doses of 1 gram in 10 c.c. of sterile distilled water.

Mercurial Dermatoses.—It is now well known that mercury may produce a variety of skin lesions somewhat resembling those produced by arsphenamin and its substitutes, but much less frequently and usually of much less severity. Almkrist[40] lately studied thirty-eight cases and classified them as simple and complicated or infective. He found that intramuscular injections of mercury salicylate was responsible for most of them, gray oil less and mercurial ointment the least.

Mercurial dermatitis may be caused by inunctions, and may be localized to the sites of application, or more generalized. Fournier states: "This dermatitis may take the form of an *erythema*, which may be diffuse or in confluent patches, extending over the whole region of inunction. This disappears in a few days, and is followed by slight desquammation. In other cases there is *mercurial eczema*, which consists in a deep red diffuse area of erythema covered with confluent vesicles, full of clear fluid at first, afterwards turbid. This is accompanied by inflammatory swelling of the skin, local heat, and intense pruritus. This generally lasts only a short time, and is followed by slight desquammation. In some cases, however, instead of a circumscribed dermatitis, a generalized eruption is produced, starting from the seat of inunction and spreading over nearly the whole skin. This is erythematous in some places and ezematous in others, and degenerates partially into an *exfoliating dermatitis*, which may last for weeks. It is sometimes accompanied by severe general symptoms, and has been described under the name of *hydrargyria febrilis maligna*. But this danger is not peculiar to the inunction method, and may occur with all the different modes of mercurial administration. Moreover, it is due to a rare idiosyncrasy."

Mercurial dermatitis most commonly occurs after the first dose of the drug, but sometimes it does not appear for several days or after several weeks. Fournier states that it is a curious fact that these eruptions are not, as a rule, accompanied by other signs of mercurial intolerance, such as diarrhea, stomatitis, etc.

Mercurial dermatitis may appear as a *simple erythema, a scarlatiniform erythema*, as an *urticaria*, an *erythema multiforme*, or, more rarely as a severe *dermatitis exfoliativa*. In other words, the lesions may be quite similar to the better known arsphenamin rashes, but usually of a milder character; indeed, as recently stated by Wright[40a] mercury may produce almost as many cutaneous reactions as arsphenamin, and it is well to bear this in mind when a patient receiving mixed treatment develops an eruption, for it may be due to either drug or to the combination.

Complicated mercurial dermatitis, as classified by Almkrist, is characterized by an irregular and very long course, often with alternate improvements and exacerbations. When the lesions become secondarily infected with bacteria, he classifies the exanthem as infected mercurial dermatitis. In the rare very acute cases of exfoliating mercurial dermatitis there may be exfoliative ileo-colitis, bronchitis, vaginitis or exfoliating conjunctivitis as in arsenical exfoliative dermatitis.

Fixed exanthema in which the outbreak is localized and recurring after each administration of mercury at the same site, may occur as described by Langer[41] Hubschmann[42] and others.

It is hardly necessary to caution against a diagnosis of mercurial dermatitis in cases of secondary syphilis showing exacerbations of skin and mucous membrane lesions after the first large dose or two of mercury; these are the

original Jarisch-Herxheimer reactions due to the influence of the drug on spirochetes and not primarily toxic mercurial exanthems. Since arsphenamin and its substitutes are now usually employed in the treatment of acute syphilis, they are not as frequently seen as ten years ago.

Fournier was of the opinion that idiosyncrasy to mercury was the primary *cause* of these exanthems. Almkrist assumes that the toxic action of mercury produces a vascular dilatation with edema by paralysis of the vasomotor nerves, followed by a proliferation of epithelium and secondary bacterial infection. It would appear, therefore, that the cause of mercurial dermatitis is exactly similar to the explanation which I have offered for arsenical dermatitis, namely, that there is a natural or acquired allergic hypersensitiveness to the metal which is expressed in the form of disturbances of the vasomotor nerves resulting in vasodilation and serous exudation which thereby intensifies the physiological vasoparetic effects of mercury. Whether or not allergic sensitiveness to bacteria from focal infections plays a part by way of the mercury stimulating or disturbing such foci with the result that an explosive allergic reaction occurs, as claimed by Stokes, Keidel and Moore in the mechanism of arsphenamin dermatitis, cannot be stated, but I do not believe that such occurs because the mercurial exanthems are so frequently engendered by doses of mercury so small that it is not reasonable to assume that they are capable of disturbing foci of bacterial infection, as at the roots of the teeth, in the tonsils or in the accessory sinuses.

The *treatment* of mercurial dermatitis is exactly the same as described for the treatment of arsenical dermatitis on page 693. In mild cases nothing is ordinarily required but withdrawal of the drug and the application of soothing lotions. In severe cases the diet must be restricted and sodium thiosulphate may be administered by intravenous injection as advised by Dennie[43] Hoffman and Scheus.[44] It is well not to resume the administration of mercury after recovery, but to continue the treatment of syphilis with the arsenicals or bismuth.

Mercurial Neuritis, Neurorecurrences and Neural Jarisch-Herxheimer Reactions.—Mercury does not involve the peripheral nerves with anything near the frequency of arsphenamin and its substitutes. Indeed, mercurial neuritis is rare in the treatment of syphilis although cases have occurred in acute mercurial poisoning as recently reviewed by Beeson.[45] Paresthesia, polyneuritis, toxic amblyopia, etc., so frequently encountered when the organic arsenicals are being administered, are almost unknown in mercurial medication. When intramuscular injections are given, nerves may be involved with referred pain to the leg and foot and even temporary paralysis of one or more muscle groups may occur.

Neural Herxheimer reactions, that is, exacerbations of lesions of the central nervous system and especially of the optic, oculo-motor and auditory nerves after the first few doses of mercury, are very exceptional occurrences and, indeed, it is a good practice to start the treatment of chronic syphilis likely to develop these lesions with a course of mercury in order to avoid them before arsphenamin or one of its substitutes is given.

Neurorecurrences, however, or the exacerbation of neural lesions upon the cessation of treatment, may occur just as in arsphenamin treatment when the restraint upon spirochetic activity is removed.

Effects of Mercury upon the Blood and General Nutrition.—When mercury is taken in toxic doses it is apparently followed by a destruction of erythrocytes producing a chloro-anemia and leukocytosis; the literature has been recently reviewed by Bessesen.[46] But otherwise mercury is to be regarded as stimulating the bone-marrow, with an increase of erythrocytes and hemo-

globin. It is very well known clinically as influencing favorably the various syphilitic anemias, although not as rapidly as arsphenamin and its substitutes.

In the treatment of syphilis when the administration of mercury is greatly prolonged or persisted in with patients who do not bear it well, there may occur languor, anemia, anorexia, fatigue and emaciation, but almost exclusively under the two following conditions: (1) when given in large doses causing gastro-intestinal disturbances; (2) when given too long, even in moderate doses. Otherwise the administration of mercury quite commonly results in an increase of weight, correction of anemia and a general improvement in health and nutrition, not so much because of the drug possesses "tonic" properties, as because of destruction of *Sp. pallida* and its products.

The bone changes of absorption and caries are likewise only observed in chronic severe poisonings; they were not uncommon in the fifteenth and sixteenth centuries as a result of barbarous methods in administration, but are very rare at the present time although caries and discoloration of the teeth and absorption of the alveolar processes are sometimes seen in severe cases of mercurial gingivitis.

Mercurial Phlebitis and Thrombosis.—When mercury is administered by intravenous injection, phlebitis may occur but not as frequently as in the administration of arsphenamin. Flumerin is said to be non-irritating to the veins even after many injections, although moderately painful swellings, lasting about twenty-four hours, have resulted from accidental perivascular injections. Cole and his associates have noted sclerosis and thrombosis in a few instances after the administration of mercurosal; mercurophen is also irritating unless well diluted—1/3 grain (0.02 gm.) in 20 c.c. of water being without irritation. The succinimid is much less irritating than the bichlorid and cyanid of mercury.

If treatment is required, it may be the same as described on page 710 for accidental arsphenamin phlebitis and peri-phlebitis. As a rule, the local application of wet dressings of a saturated solution of magnesium sulphate for a day or two is all that is required.

Pain, Nodosities and Abscesses from Intramuscular Injections of Mercury.—Intramuscular injections of mercury almost always produce some pain, although the degree varies greatly in different individuals. By incorporating an anesthetic, as 0.1 c.c. (2 or 3 drops) of a 2-per cent. solution of butyn, the immediate pain is prevented, but not the late pain coming on after a day or two. In a general way, the pain produced by mercury is greater than that produced by bismuth; this is certainly true of the insoluble salts of both metals.

As a rule, the pain from intramuscular injections of the soluble salts of mercury comes on in an hour or two or several days after injection; in the latter cases it is more acute and continuous with tenderness of the whole region. In my experience, the bichlorid and biniodid of mercury have produced more pain than mercurosal; mercurophen produces so much pain that it cannot be administered at all by intramuscular injection. Flumerin has not been employed by this route of administration.

Intramuscular injections of the insoluble salts of mercury also produce pain and especially injections of calomel. Gray oil is possibly a little less painful and the salicylate is much less irritating. The degree of pain and complaint varies, however, with different patients, but in the majority, a series of calomel injections in dose of 0.05 gm. (about 1 grain) cannot be given, whereas it is usually possible to give a series of injections of the salicylate in the same dose. In rare instances fever and such symptoms as malaise, ano-

rexia, headache, insomnia, and weakness accompany the local reaction and confine the patient to bed for several days.

As a general rule, very little can be done in the way of treatment. Hot or cold applications of saturated solutions of magnesium sulphate in water may be tried if the patient is confined to bed. Otherwise mild counter-irritation by occasional applications of diluted tincture of iodin may afford relief.

The nodosities from injections of the soluble salts usually vary in size from a filbert to a walnut. They are rounded, rather diffuse, hard, and sensitive to pressure. They often disappear in a few days, but sometimes persist for weeks. Sometimes they give rise to a hyperesthetic state of the whole region in which they are situated, especially when they are multiple. After injections of calomel they may persist for weeks and months and occur in about two thirds of the cases. Gray oil nodosities are also likely to persist for long periods of time; the salicylate is less likely to produce them although they occur and often persist with some tenderness for weeks and months; in the subcutaneous tissues I have known them to persist for several years due to fibrous tissue encapsulation.

Nodosities may be treated by occasionally painting the skin with tincture of iodin for counter-irritation and gentle massage and pinching to encourage complete absorption of the mercury, inflammatory exudate and fibrous tissue. Sometimes, and especially when located in the subcutaneous tissue, they may be removed surgically.

Sloughing rarely follows injections of mercury as may occur after injections of arsphenamin. When mercury is given subcutaneously, however, large sloughs may develop and especially if secondary bacterial infection occurs. Abscesses are likewise rare; in Fournier's experience they occurred only in 19 injections in a series of 1410 injections of calomel. They are not usually caused by bacterial infection but are sterile abscesses due to chemical liquefaction necrosis by the mercury with dark chocolate colored fluid contents. They must be treated by incision and evacuation under aseptic precautions to prevent secondary injection.

Hemorrhages from accidental rupture of large blood-vessels in intramuscular injections are relatively rare; not infrequently, however, small superficial veins are punctured during the passage of the needle but without anything more than slight bleeding easily checked by pressure.

Pulmonary Embolism from Intramuscular Injections.—As previously stated, colloidal suspensions of calomel and of the metal mercury itself may be injected intravenously without danger of embolism if the particles are not much larger than erythrocytes as determined by microscopical examination. But when oily suspensions of calomel, salicylate or gray oil are accidentally injected and especially rapidly injected into a vein during the course of intramuscular injections, oil embolism of the lungs may result. Immediately, or a short time after injection, there is sudden dyspnea, rapid respirations, spasmodic cough, pain in the thorax, and even syncope; then on the next or following days, slightly blood stained expectoration, and the signs of localized patches of congestion and exudation. Recovery is the rule under simple treatment of rest in bed with sedatives.

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CHAPTER LIV

PHARMACOLOGY OF BISMUTH IN RELATION TO METHODS OF ADMINISTRATION AND THE TREATMENT OF SYPHILIS

Since compounds of bismuth have been employed in the treatment of syphilis only during the past year or two, our knowledge of their pharmacology is quite incomplete and especially in relation to administration by intramuscular injection. It is true that for many years various salts of bismuth have been employed in the treatment of gastro-intestinal diseases by oral administration, in the form of dusting powders and Beck's paste, for surgical purposes and for aiding the preparation of roentgenograms; but

otherwise the therapeutic use of bismuth has been quite limited although the toxicological effects have been carefully studied and especially in relation to the use of these compounds in surgical treatment.

Much that has been stated on the absorption and elimination of mercury in Chapter LII is applicable to bismuth compounds, although the latter are much less volatile and are absorbed much more slowly from the gastro-intestinal tract. While Klauder[1] has employed daily inunctions of 0.5 to 2.0 gm. of bismuth trioxid in the treatment of syphilitic patients presenting cutaneous lesions, only feeble curative effects were observed, probably largely due to the fact that bismuth is less volatile than mercury which reduces the amount absorbed by inhalation of vapors or the swallowing of condensed vapors in the saliva. Absorption from the gastro-intestinal canal is likewise so slow and uncertain that this route of administration is not employed in the treatment of syphilis; intravenous injections of soluble salts, like potassium and sodium tartrobismuthate, have proven somewhat too toxic and dangerous, although bismuth hydroxid in colloidal suspension has been widely employed abroad in dose of 0.010 gm. (about $\frac{1}{6}$th grain) by intravenous administration. At the present time the intramuscular injections of potassium tartrobismuthate, sodium and potassium tartrobismuthate, bismuth oxid or other compounds suspended in vegetable oils are mostly employed, and I will therefore discuss the absorption and elimination of bismuth principally from the standpoint of this route of administration.

Absorption of Bismuth with Special Reference to Intramuscular Injection.—As stated in Chapter XXV, a large number of soluble and insoluble bismuth compounds have been tried in the treatment of experimental syphilis of the rabbit, including the precipitated metal and bismuth hydroxid in suspension, bismuth ammonium citrate, lactate, subgallate, oxyiodgallate quininbismuth iodid, trioxybismuthobenzoate and bismuth trioxid, but potassium tartrobismuthate, sodium tartrobismuthate and a quartenary compound known as sodium and potassium tartrobismuthate have been found by Sazerac and Levaditi and others least toxic and especially suitable for intramuscular injection either in isotonic solution or in suspension in a vegetable oil.

Of course, when bismuth is injected intravenously, either in the form of solutions of the water soluble salts like the tartrobismuthate or the insoluble compounds as bismuth hydroxid in colloidal suspension, the question of absorption is settled, since the drug is placed at once in the blood stream.

Studies upon the rapidity of absorption of bismuth into the blood after intramuscular injections do not appear to have been made, but the results of experiments in the treatment of syphilis of the testicles of rabbits have shown me that solutions of sodium and potassium tartrobismuthate are rapidly absorbed and probably the rate is comparable to the absorption of such soluble salts of mercury. It is well known, for instance, that absorption of soluble salts from the intestines or ulcerated skin occurs so rapidly that bismuth may be found in the urine within 10 to 15 hours after administration.

Suspensions of sodium or potassium tartrobismuthate or other bismuth salts in oil are absorbed more slowly and especially when injected in paraffin oil. Levy-Bing, Belgodère and Auclair[2] have studied the rate of absorption by means of roentgenograms of 0.015 gm. doses of bismuth hydroxid, washed with alcohol, suspended in olive oil, sterilized and injected every 2 to 7 days, and found that absorption was ordinarily quite rapid but varying according to the site of injection, compression and massage, etc. Fritz[3] has studied the absorption of "bismoluol," a dipotassium-bismuth preparation, administered in olive and paraffin oils. In the former menstrum absorption was

usually complete in a week; but in the latter, absorption was very irregular and retarded, because paraffin oil remains unchanged in the tissues forming a protective coating over the medicament whereas the vegetable oil is saponified by the alkaline tissue juices and readily absorbed. Beinhauer and Jacob[3a] have injected six preparations of bismuth into the gluteal muscles followed by roentgenological examinations for absorption with the following general results: "neotrepol and bicreol were more widely diffused and more quickly absorbed than most preparations. Iodobismuthate of quinine was most quickly absorbed but iodidism resulted in several cases. Bismudol was readily absorbed and very well tolerated. Potassium-bismuth-tartrate and bismuth salicylate were slowly absorbed and we found that the cumulative action must be considered when using these preparations. When using the quickly absorbable preparations, the toxic effect must be borne in mind."

It would appear, therefore, that in the treatment of syphilis preference is to be given the soluble salts of bismuth administered by intramuscular injection in doses of 0.1 to 0.2 gm. in a menstrum of sterile vegetable oils, like almond or olive oils, in amounts of 1 or 2 c.c. Under these conditions cumulative effects are not likely to occur and absorption is ordinarily complete in 5 to 7 days. As I shall discuss with more detail in the next chapter some tissue necrosis occurs about the deposited drug in the muscles with well-marked leukocytic infiltrations, but nevertheless absorption is quite rapid. The insoluble compounds, however, like the precipitated metal, are absorbed more slowly and in a degree comparable to the absorption of calomel, but apparently faster than the absorption of gray oil as indicated by the studies of Levy-Bing and his associates referred to above.

Absorption may also occur after the oral administration of the subnitrate and subgallate; according to Dorner and Weingartner,[4] small amounts of these basic salts are dissolved in the acid gastric juice followed by absorption. As shown by Kocher and numerous other surgeons, absorption may also occur when bismuth powders are applied to wounds or in the form of Beck's paste; quite a series of poisonings have been recorded, although, in some of these at least, the effects have been caused by the absorption of nitrites from impure preparations of bismuth subnitrate.

Mechanism of the Absorption of Bismuth.—Little is known of the mechanism of the absorption of bismuth. It is commonly stated that albuminates are formed in the tissues, similar to the albuminates of mercury, by dissociation of bismuth ions, which are rendered soluble in the tissue fluids and absorbed. I have observed that solutions of potassum tartrobismuthate and sodium tartrobismuthate in saline solution form precipitates somewhat slowly with serum, which, like the abuminates of mercury, are insoluble in saline solution; but doubtless these abuminates are rendered sollube in the tissue fluids by chemical reactions followed by absorption in the same manner as the absorption of the albuminates of mercury.

Absorption of the metal itself and of its insoluble salts also occurs by phagocytosis, as shown by the studies of Meyer and Steinfield,[5] Schmelzer,[6] and Langhaus,[7] on absorption from the intestines and wounds; Mayer and Baehr[8] have also demonstrated bismuth in phagocytic cells by a microchemical method, and doubtless phagocytic absorption plays an important rôle in the absorption of both organic and inorganic compounds, the phagocytized particles later undergoing solution in the cells or in the tissue fluids.

Distribution of Bismuth after Absorption in Relation to the Treatment of Syphilis.—The small amount of data available from chemical studies of the internal organs following fatal intoxications with bismuth, indicate that wide distribution occurs, the results being quite similar to those observed in

investigations upon the distribution of mercury. Analyses of the organs of human subjects, succumbing to poisoning following the application of bismuth to wounds and its use in paste, have shown its presence in the buccal mucosa, salivary and cervical glands, liver, spleen, kidneys and the walls of the gastro-intestinal canal. Dalché and Villejean,[9] for example, administered the subnitrate to dogs by subcutaneous injection followed by chronic poisoning and analyses of the organs of one animal showed that the concentration was least in the liver, but, owing to its large size, this organ contained most of the drug and, as will be shortly discussed, is actively concerned in its elimination in the bile. Similar results were observed in a case of fatal poisoning following the injection of 3 ounces of a 25 per cent. chemically pure paste of bismuth reported by Mayer and Baehr.

Of considerable interest in relation to the treatment of neurosyphilis is whether or not bismuth is to be found in the tissues of the central nervous system and cerebrospinal fluid. Aubry,[10] employing a modification of Léger's method, states that he was able to detect bismuth in a dilution of 1:600,000 and that with this method bismuth was found by Démelin and himself in the cerebrospinal fluid of patients treated with intramuscular injections of 0.2 gm. of "trepol" which is sodium and potassium tartrobismuthate. Jeanselme, Delande and Tenis[10a] employing the same method, which they state can detect one part of bismuth in a million, tested the cerebrospinal fluids of 31 cases of syphilis following intravenous injections of bismuth but with negative results in all.

It is highly probable, therefore, that bismuth has much the same distribution as mercury and lead and is to be especially found in those organs mostly concerned in its elimination, as the liver, kidneys, salivary glands, gums and colon. Syphilitic lesions and especially chronic or tertiary lesions of the skin and mucous membranes, periosteum and bones, as well as of the central nervous system and other internal organs, are known to show therapeutic improvement under bismuth administration and especially some types of neurosyphilis refractory or resistant to mercury and the organic arsenical compounds; these therapeutic results indicate, therefore, a widespread distribution of bismuth.

Elimination and Retention of Bismuth in Relation to the Treatment of Syphilis.—After absorption some bismuth is apparently stored in the liver and other organs, but most is gradually eliminated in the urine and bile; elimination also occurs by the salivary glands, buccal mucosa and probably the entire gastro-intestinal tract and especially from the first portion of colon. According to Fournier and Guénot, traces are to be found in the sweat; probably none is eliminated through the lungs. Apparently small amounts are also eliminated in the milk of lactating women, the whole mechanism of elimination being apparently quite similar to that of mercury.

Elimination of Bismuth in the Urine.—Even when given by mouth some bismuth may be found in the urine; Mayer and Baehr mention that Kuttner found 0.023 gm. in the urine of a case of gastric ulcer six days after the oral administration of one ounce of bismuth carbonate. Levaditi[12] states that bismuth appears in the urine within eighteen to twenty hours after the intramuscular injection of sodium and potassium tartrobismuthate, and that it continued to be eliminated in the urine for twenty to twenty-five days after a course of treatment consisting of a total administration of 2.0 to 2.5 gm. of this substance. Steinfield and Meyer in 1886 noted its presence quite constantly in the urine after subcutaneous injections of bismuth oxid and in 1818 Orfila observed the same after the oral administration of the subnitrate. Sometimes the urine when voided is discolored or becomes so after standing, which in the latter instance is due, according to Démelin, to the formation of sulphids of bismuth by the bacteria present. It would appear, therefore, that the kidneys are the main enunctories of bismuth, as is true of mercury and lead, and for this reason due care must be exercised, as stated by Higgins,[13] in the administration of bismuth for any

purpose; and I believe that this should apply to the treatment of syphilis with intramuscular injections of the tartrobismuthates, although I have frequently injected potassium tartrobismuthate in dose of 0.1 gm. in 2 c.c. of sterile almond oil once a week for six to twelve weeks, when traces of albumin with casts resulting from mercurial treatment have gradually disappeared from the urine.

Elimination of Bismuth in the Feces.—Following subcutaneous injections of bismuth oxid, Steinfield and Meyer in 1886 found bismuth in the bile, and since then several investigators have shown that considerable amounts are eliminated by this route. Apparently the liver is an important storage depot for the drug and likewise an important organ of elimination. The presence of bismuth in the feces is largely due, therefore, to elimination in the bile, but in addition some bismuth is eliminated in the mouth which is swallowed, as well as probably along the entire gastro-intestinal tract. In poisoning, the colon shows the most marked changes with blackish areas of ulceration; Steinfield and Meyer have stated that these effects are due to the precipitation of bismuth brought to the mucosa for elimination in the lymphatic and vascular channels by the formation of bismuth sulphid from the hydrogen sulphid in the colon and that these precipitates produce embolism, stasis and necrosis. The same explanation has been offered for the blackish deposits in the gums. Under these circumstances ulcers may be produced as the result of the stasis and direct irritating effects of the sulphid and the black discoloration of the feces is believed to be due in part at least, to the presence of these sulphid compounds. Schmelzer, however, has questioned this view, because similar pathological changes may be produced apparently in the absence of hydrogen sulphid in the colon of some animals like the goose. It is possible that a portion of the black deposits in the feces are oxids of bismuth, although there is but little oxygen available in the colon for their production.

However, there cannot be any doubt that bismuth is actively eliminated by the liver in the bile and by at least some portions of the alimentary tract, and especially the buccal mucosa and colon. It is also possible that some is eliminated in the pancreatic juices as in the case of mercury. Probably a portion eliminated in the bile and saliva is re-absorbed lower down, as may occur in the case of mercury with a repetition of the cycle. When bismuth is administered by mouth, of course the largest portion may be passed in the feces unabsorbed. As a general rule the feces are black, due to the formation of sulphids and oxids, but in some cases of enteritis the bismuth may be passed almost unchanged. Whether this denotes an absence of hydrogen sulphid or a change in the bacterial flora cannot be stated.

Elimination of Bismuth in the Milk.—Of special interest in relation to the treatment of syphilis of nursing infants by administration of drugs to the mother, is the elimination of bismuth in the milk. According to Cabot,[14] Henry and Chevalier showed in 1839 that bismuth may be eliminated in the milk of goats and Schmelzer states that Lewald also found bismuth in the milk of women. It is highly probable, therefore, that bismuth administered to nursing women may be eliminated in small amounts, at least, in their milk, but I do not know at this writing of any clinical observations upon the influence exerted upon syphilitic infants during the intramuscular injections of the tartrobismuthates to the nursing syphilitic mothers.

Retention of Bismuth.—Undoubtedly bismuth tends to become stored in some of the internal organs and especially the liver. Very probably other organs, as the kidneys and gastro-intestinal mucosa, likewise store small amounts, as in the case of mercury. Just what may be the capacity for storage in the different organs cannot be stated; nor do we have any information regarding the balance or equilibrium between storage and elimination as worked out by Lomholt for mercury. Very probably, however, all that has been determined for mercury is applicable in a general manner to bismuth, although, owing to a lower toxicity of bismuth and especially the tartrobismuthates employed in the treatment of syphilis, it is likely that the liver, kidneys and other organs can store larger amounts without apparent injury than is the case with mercury; likewise, it is probable that the blood may carry more than it may of mercury.

Nothing is known of the therapeutic activity of stored bismuth in syphilis except to state that in all probability it possess some value since Kolle[14a] has shown that when bismuth was injected subcutaneously in the ears of rabbits, the drug prevented the development of testicular syphilitic lesions until the deposit was removed by amputation, following which lesions usually developed; in other words it would appear that local deposits of bismuth may at least prove spirochetostatic and the same is probably true of depots of mercury. As is probably true of mercury, some of the stored metal is doubtless being constantly re-absorbed and may exert some therapeutic effect before elimination occurs. For this reason, curative effects may continue for some time after the cessation of intramuscular injections of the tartrobismuthates in syphilis and, indeed, clinical experience indicates that this is the case.

Mechanism of Therapeutic Activity of Bismuth in Syphilis.—The subject of the mechanism of curative activity of bismuth in syphilis has been already

discussed in Chapter XXV. It is highly probable that this mechanism is essentially similar to the spirocheticidal activity of mercury and largely dependent upon the ease with which the bismuth ions become dissociated. Very probably the toxicity of bismuth for both the cells of the host (organotropism) and for *Spirochæta pallida* (parasitropism) depends upon the bismuth ions, as there is no evidence to show that new compounds are produced by processes of oxidation or reduction as in the case of the organic arsenical and antimonyl compounds; nor is there any evidence to show that bismuth increases the production of spirocheticidal antibodies by direct stimulation of the antibody producing tissues.

Just how much bismuth is required in the blood and tissue fluids to exert spirocheticidal effects is not known. As stated in Chapter XXVIII, I have found that 1:80 solutions of the tartrobismuthates in serum and tissue fluids are required to bring about the destruction of *Sp. pallida in vitro* in an exposure of 30 minutes at 37°C., but whether more or less than this concentation is required *in vivo* cannot be stated. The single minimal curative dose of the tartrobismuthates in acute testicular syphilis of rabbits is about 0.05 gm. per kilogram by intramuscular injection, and if all of this were absorbed before elimination occurred would probably yield a dilution in the blood of approximately 1:1000 per kilogram of body weight. It is highly probable that this concentration is sufficient for the destruction of spirochetes in the blood, while in the tissues the drug is probably cumulative in action until a concentration is reached at which spirocheticidal effects are produced; in in this manner, the administration of small doses of bismuth results in the accumulation in the tissues of curative amounts, since elimination is always slower than absorption when the tartrobismuthates are given human beings or the lower animals in amounts of approximately 0.002 to 0.005 gm. per kilo every 5 to 7 days which corresponds to about 0.1 to 0.2 gm. for the average human adult.

The spirocheticidal activity of bismuth being at least four times greater than mercury permits the administration of sufficient amounts to bring about more rapid curative effects in acute early syphilis than is ordinarily the case with mercury and in such cases I believe that bismuth is the remedy of choice if for any reason arsphenamin, neoarsphenamin or their substitutes cannot be given. In my opinion bismuth probably accumulates in syphilitic tissues to a greater extent than in non-infected tissues and in this manner the amount of bismuth ions reaches a spirocheticidal concentration in these areas, even though the amount in the blood may be below this level. Of course, the drug is being constantly eliminated, but by proper dosage it is possible to maintain the spirocheticidal concentration in the spirocheta infected tissues without damaging the kidneys, liver and gastro-intestinal tract with excessive amounts.

Just how bismuth destroys *Spirochæta pallida* is not known. It may be that the metal unites with and precipitates the proteins of the parasites which results in their death, followed by digestion by enzymes from the parasites themselves, the tissue fluids or tissue cells. This mechanism is just as obscure as the whole subject of the mechanism of the destruction of microparasites by germicides.

Other Pharmacological Effects of Bismuth.—Aside from studies upon the absorption and elimination of bismuth summarized above and the toxicological effects to be considered in the succeeding chapter, little more is known of the pharmacology of bismuth. We do not have any information, for example, concerning its influence upon blood pressure, the chemistry of the blood, kidney function, etc., as discussed in Chapter XLIII in regard to

arsphenamin and neoarsphenamin. Fournier and Guénot noted temporary polyuria and albuminuria in a few cases treated with intramuscular injections of the tartrobismuthates, but never any persistent disturbance of renal function; evidently the diuretic effect is similar to that sometimes produced by mercury. No doubt, if bismuth becomes widely employed in the treatment of syphilis, studies in these subjects will be made owing to their very useful and practical importance.

Mention may be made here of the blood changes produced by bismuth as reported by Betz.[15] Examinations were made by 25 cases in the primary and secondary stages, before and after each injection. As a general rule a mild and temporary degree of leukocytosis followed each injection, especially in the beginning of treatment, due to an increase of the neutrophils and eosinophils and in the later stages to an increase of lymphocytes. In some cases the erythrocytes were increased, while in a few, however, there were evidences of erythrocytic destruction with anisocytosis but with no evidences of poikilocytosis. Evidently these effects upon leukocytes, erythrocytes and hemoglobin were due primarily to the action of bismuth on the bone marrow, which may temporarily stimulate its functions or, in large doses, exert a toxic depressive effect with blood destruction.

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CHAPTER LV

TOXICOLOGY OF BISMUTH IN RELATION TO METHODS OF ADMINISTRATION AND THE TREATMENT OF SYPHILIS

From the standpoint of the treatment of syphilis with bismuth most interest is to be placed in the toxicological effects produced by intramuscular and intravenous injections of various organic and inorganic salts and especially of the tartrobismuthates. A knowledge of these untoward effects is imperative for the treatment of syphilis with bismuth and these compounds cannot be given by rule since the toxic effects vary in different patients and some individuals are more susceptible than others.

General Toxicology of Bismuth.—Instances of chronic poisoning from the application of dusting powders and salves of bismuth subnitrate to wounds are not lacking, since first described by Kocher[1] in 1882; Mayer and Baehr[2] have collected 42 such cases up to 1912, 13 of them being fatal intoxications. In addition to these chronic poisonings a number of cases of acute poisoning first reported by Benneke and Hoffman[3] in 1906, have been recorded following the internal administration of bismuth subnitrate for roentgenographic purposes. In some of these at least, poisoning has been due to the absorption of contaminating nitrites instead of bismuth and since the adoption of the carbonate of bismuth and other precautions for *x*-ray work the danger of poisoning from these has been removed. Chronic bismuth poisoning has also followed the use of Beck's paste, Mayer and Baehr having collected 19 cases from the literature and adding 3 of their own, one of which had a fatal outcome following the injection of three ounces of a 25-per cent. bismuth paste into a sinus for roentgenographic purposes.

Undoubtedly the widespread use of the tartrobismuthates, bismuth hydroxid and other organic and inorganic salts in the treatment of syphilis will result in greatly renewing clinical interest in bismuth poisoning and, since it is essential for the physicians to be acquainted with the lesions and symptoms of this intoxication, the subject is here considered from the standpoint of intramuscular and intravenous routes of administration in relation to the treatment of syphilis.

Chemistry of Bismuth Compounds in Relation to Toxicity and Therapeutic Activity.—During the past four years a large number of new compounds of bismuth have been proposed for the treatment of syphilis and especially organic salts, but little is known of the relationship of their chemical constitution to toxicity for the host (organotropism) and toxicity for the spirochetes (parasitropism). The ordinary salts, as the subnitrate and subgallate, have been found too inert, although they have not been extensively employed by intramuscular injection. At the present time the tartrobismuthates of sodium or potassium and colloidal suspensions of the hydroxid, are most popular for the treatment of syphilis. Insofar as chemical constitution is concerned, I believe that the ease and rapidity with which the bismuth ions are dissociated after administration will prove the important factor as is apparently true of mercury compounds; of importance likewise are the amounts of bismuth in the compounds and their solubility in the tissues and absorbability after administration. Doubtless further chemical research will result in evolving compounds of lesser toxicity and especially by intravenous injection, but these are likewise apt to be of lesser therapeutic activity since the reduction in toxicity is likely to be based upon a decreased power of dissociation of bismuth ions, which I believe is the important factor in relation to both organotropism and parasitropism.

Toxicity of Bismuth for the Lower Animals by Intramuscular and Intravenous Administration.—Before discussing the tissue changes and symptoms of bismuth poisoning in human beings, it may be well to briefly review the effects observed among the lower animals in experimental intoxications. As far back as 1886, Steinfield and Meyer[4] injected bismuth oxid subcutaneously into frogs, rabbits, cats and dogs and produced two types of poisoning; about one year later, Dalche and Vallejean[5] observed similar results following subcutaneous injections of bismuth subnitrate into dogs. These experiments showed that sufficient amounts of bismuth could be absorbed from the subcutaneous tissues to produce toxic lesions and symptoms and confirmed the clinical observations of Kocher and others.

Since the introduction of bismuth into the treatment of syphilis by Sazerac and Levaditi in 1921, several investigators have studied the toxicity of various organic and inorganic compounds for the lower animals, and chiefly the rat, guinea-pig and rabbit to determine the relative toxicity of these compounds by intramuscular and intravenous injection, as based upon the smallest amounts producing immediate death (*dosis lethalis*) or the largest amounts tolerated over one or two weeks (*dosis tolerata*). No attempt was made in tests of this kind to reproduce the characteristic symptoms of bismuth poisoning in man, but they are not without value as indices of the relative toxicity of different compounds by different routes of administration and likewise reproduce for histological study the various tissue changes that may be engendered.

The results of some tests of this kind have already been briefly referred to in Chapters XV and XXV. Most interest has been placed in potassium and sodium tartrobismuthate, because this salt has been especially recommended by French physicians for the treatment of human syphilis; recently Pacella[5] reported the results of a series of studies upon the toxicity of this tartrobismuthate for frogs, guinea-pigs and rabbits. Levaditi and Sazerac have found that the minimal toxic dose of water soluble sodium and potassium tartrobismuthate (luatol) for rabbits by intravenous injection was about 0.010 grams per kilo and about 0.050 grams per kilo by intramuscular injection. Myers and Corbitt,[7] however, found that the maximum tolerated dose of "luatol" for white rats by intravenous injection was 0.036 grams per kilo for a period of seven days and that compounds prepared by them were tolerated in dose of 0.050 grams per kilo by intravenous injection. Of course, it is to be expected that differences in the percentage of bismuth in the compounds tested, concentration of the solutions, rapidity of injection, kind of animal employed, condition of the animals, etc., would be factors modifying the results of toxicity tests by different investigators as discussed more fully in Chapter II. Myers and Corbitt, however, employed the same technic as is used for determining the toxicity of arsphenamin and neoarsphenamin and I have employed the same method in my toxicity tests with water soluble tartrobismuthates. In my experiments white rats were injected intravenously with graded amounts of the tartrobismuthates in solution, the rate of injection being 1 c.c. in two minutes, etc., exactly as described in Chapter II for the testing of the organic arsenical compounds. With these tests the minimal lethal and maximum tolerated doses were ascertained. In addition, I have determined the maximum tolerated doses of the water-soluble tartrobismuthates for rabbits by the intravenous injection of multiple small doses as one would employ for the treatment of syphilis; also, the maximum tolerated doses of water-soluble and oily suspensions of the tartrobismuthates by intramuscular injection of multiple small doses comparable to those administered by this route for the treatment of syphilis.

In these experiments "luatol" or the water-soluble sodium and potassium tartrobismuthate and "trepol," the suspension of the same compound in a vegetable oil, both of French manufacture, were employed; sodium tartrobismuthate, potassium tartrobismuthate and bismuth trioxid were kindly prepared and furnished me by Dr. George W. Raiziss.

(*a*) *The Minimal Single Lethal Doses of the Tartrobismuthates by Intravenous Injection.*—As previously stated, white rats of 100 to 150 grams kept under standard conditions were employed, the injections being given in a femoral vein at the rate of 1 c.c. per two minutes of 1 per cent. solutions; the smallest amounts producing immediate or almost immediate and fatal effects were approximately as follows:

Sodium and potassium tartrobismuthate (41.5% Bi).	0.090 grams per kilo
Sodium tartrobismuthate (59.08% Bi).....	0.090 grams per kilo.
Potassium tartrobismuthate (61.32% Bi).....	0.095 grams per kilo.

(*b*) *The Maximum Single Tolerated Doses of the Tartrobismuthate by Intravenous Injection.*—At the same time, the maximum single tolerated doses of these compounds for white rats by intravenous injection were determined, the results being approximately as follows on the basis of survival for a period of fourteen days:

Sodium and potassium tartrobismuthate.....	0.024 grams per kilo.
Sodium tartrobismuthate.....	0.018 grams per kilo.
Potassium tartrobismuthate.....	0.020 grams per kilo.

It will be noted that the quartenary compound sodium and potassium tartrobismuthate was tolerated in slightly larger amounts than the sodium or potassium tartrobismuthates, probably because the former contained less bismuth than the latter two compounds. Of course, the doses mentioned are not absolute as slightly varying results were observed due to variations in the susceptibility of different rats of approximately the same weight and general condition on the same diet. As stated in Chapter II, it is not possible to render the results of biological tests of this kind mathematically correct, but the above values are approximately correct and state the average toxicity of these compounds by intravenous injection. It is to be noted in passing, however, that the tartrobismuthates are from three

to four times less toxic than some of the soluble mercurials, as the bichlorid and benzoate; from the standpoint of absolute toxicity based upon tests of this kind, there should be no objection to the intravenous injection of the soluble tartrobismuthates in the treatment of syphilis in dose of 0.010 grams per 60 kilos or 132 pounds, which is approximately one-sixth of a grain, ten to twenty times less than the amount injected intramuscularly, and at least 100 times less than the maximum tolerated dose by intravenous injection per kilo of body weight if the tissues of the rat and human being are equally susceptible.

(*c*) *The Maximum Tolerated Doses of the Tartrobismuthates by Repeated Intravenous Injections.*—Of more interest from the standpoint of intravenous therapy have been the results of multiple intravenous injections in rabbits. These animals received increasing doses of 0.0005 to 0.008 grams per kilo every day until fatal intoxication occurred. The following number of injections were required to produce emaciation, nephritis and death:

Sodium and potassium tartrobismuthate (luatol), 39 to 43 injections or a total of 0.133 to 0.197 grams per kilo.

Sodium tartrobismuthate, 38 to 40 injections or a total of 0.117 to 0.149 grams per kilo.

Potassium tartrobismuthate, 39 to 40 injections or a total of 0.133 to 0.149 grams per kilo.

(*d*) *The Maximum Tolerated Doses of the Tartrobismuthates and Bismuth Trioxid by Repeated Intramuscular Injections.*—In the treatment of syphilis, the soluble tartrobismuthates dissolved in water or the insoluble salts suspended in a vegetable oil are ordinarily administered in dose of 0.2 grams by intramuscular injection every 5 days for 10 to 15 doses or until 2 to 3 grams have been given. For the adult of average weight this dose corresponds to 0.004 grams per kilo or a total of about 0.05 grams per kilo. Guinea-pigs, however, have withstood a series of 16 to 18 injections at intervals of 3 to 4 days of sodium and potassium tartrobismuthate in water (luatol) and suspended in oil (trepol) until as much as a total of 0.224 grams per kilo had been given without succumbing although loss in weight and pathological tissue changes in the internal organs were produced. Similar results were observed with sodium tartrobismuthate dissolved in water and suspended in olive oil; also potassium tartrobismuthate in water and olive oil and bismuth trioxid suspended in olive oil.

As a general rule, however, the solutions in water were slightly more toxic and doubtless were more rapidly absorbed than suspensions in olive oil; very probably the rate of dissociation is the same after absorption occurs and differences in toxicity are to be ascribed primarily to variations in the rate of absorption. Suspensions of bismuth trioxid, however, were slightly less toxic even though this compound contains a higher percentage of metallic bismuth, due primarily to slower absorption and dissociation of this compound as compared with the water-soluble tartrobismuthates.

Toxicity of Bismuth Compounds for the Lower Animals in Relation to Toxicity for Man.—While sufficiently extensive studies on the comparative toxicity of the organic and inorganic compounds of bismuth for the lower animals have not been made to warrant definite statements, yet data at hand indicates that the toxic effects of intramuscular injections are in relation to the amount of bismuth in the compound, the rate of absorption and probably the degree of dissociation of the bismuth ions. As is true of the mercurials, those compounds containing most bismuth are in general terms the most toxic, but the relation is not constant since a compound containing less bismuth may still be more toxic if it is more rapidly absorbed. Comparative toxicity, therefore, is a matter of absorption and dissociation of the bismuth regardless of whether the compound is a finely divided preparation of the metal itself, an inorganic or organic salt. Rate of absorption and dissociation from the muscles or subcutaneous tissues are in turn not only influenced by the menstrum employed, but likewise by the rate of solubility in the tissue juices as well as by the degree of local irritation; compounds eliciting marked inflammatory reactions are probably not absorbed as rapidly as those which do not.

When a bismuth compound is injected intravenously other factors enter into the mechanism of toxicity in addition to that of the rate of dissociation of bismuth ions; some of these factors are agglutination and hemolysis *in vivo* with the possibility of production of emboli, which I will discuss more fully later in this chapter. Here it may be stated, however, that while the experiments summarized above indicate that the soluble tartrobismuthates may be

given intravenously yet the injection of these or of colloidal preparations of bismuth hydroxid may elicit immediate reactions in the blood tending to increase the chances of untoward toxic reactions in human beings.

Toxicity tests in the lower animals are of distinct value, but do not show all the toxic phenomena that may be observed in human beings; for example, they may fail to show salivation and gingivitis in chronic intoxications and cough, thoracic oppression, general muscular pains, local pain at the site of injection, etc., which may develop in human beings treated with bismuth by intravenous injection.

Pathological Tissue Changes Produced by Bismuth in the Lower Animals.—Toxicity tests with the lower animals have aided greatly in a study of the histological changes produced by bismuth and no chemotherapeutic study can be regarded as complete that does not include an investigation of the possible tissue changes that a drug may produce and especially in the chief organs of elimination as the liver and kidneys, even though toxic symptoms are not elicited or demonstrable.

Bismuth is no exception to this rule and the pathological changes that may be produced in the physiologically important organs are of paramount interest and value, especially those that may be produced by the repeated injection of doses per kilo of weight comparable to those given human beings. Investigations of this kind are especially valuable for teaching us the minor changes that may occur before manifest lesions and symptoms are developed and tests among the lower animals are of value in this connection even though conditions in the syphilitic human subject may be altered and different by reason of additional pathological changes engendered by *Spirochæta pallida*.

Lucke and Klauder[8] have studied the changes in the kidneys and liver of rabbits following the administration of single lethal doses of the tartrobismuthates and bismuth trioxid with the general result that *renal injuries* involved chiefly the epithelium of the convoluted tubules. These showed all types of degeneration, ranging from severe cloudy swelling to extreme necrosis and marked calcification. The glomeruli did not appear primarily damaged, and often were found to be well preserved in the midst of completely necrotic tubules. The glomerular capillaries frequently contained partly conglutinated and sometimes hyalinized masses of erythrocytes. The glomerular spaces were usually free from foreign material. The *lesions in the liver* were much less conspicuous. There was usually present a definite cloudy swelling, with evident nuclear degeneration, and the appearance of minute fat droplets. Occasional small foci of necrosis flooded with erythrocytes and invaded by a few mononuclear and polynuclear leukocytes were found in most animals.

These effects were, therefore, quite similar to those produced by mercury (Chapter LIII), although the doses were very large being equivalent to from 9.0 to 30.0 grams per kilos or 132 pounds by intramuscular injection and from 0.6 to 1.8 grams by intravenous injection or at least 50 to 200 times larger than the amounts administered to human beings at one time. Kollert, Strasser and Rosner[9] injected "trepol" (an oily suspension of sodium and potassium tartrobismuthate) into rabbits and the kidneys showed a swelling of the tubular epithelium causing complete obstructions in some cases. There was also exfoliation of finely granular epithelial cells with cylinder formation producing in short, a type of degenerative lesion similar, in the opinion of the author, to the mercurial nephrosis described by Volhard and Fahr.

In connection with the toxicity tests with the tartrobismuthates and bismuth trioxid employing rats, guinea-pigs and rabbits described above, Dr. Lucke and myself have also made macroscopic and microscopic studies of the principal organs and the results have been as follows:

(*a*) The intramuscular injections of guinea pigs every 3 to 4 days with 0.010 to 0.013 grams per kilo for 11 to 17 doses, corresponding to 0.6 to 0.8 gm. per 60 kilos or approximately

3 to 4 times the amounts given at one time to human adults, produced relatively slight histological changes. In the *kidneys* the cells of many of the convoluted tubules were swollen, very granular, vacuolated, anuclear, and desquammated, but massive necroses were not found. The glomeruli were practically normal. The *liver* cells, in general, were in a state of mild cloudy swelling and very occasionally small foci of necroses were encountered. These effects were produced by all of the compounds studied, namely, sodium and potassium tartrobismuthate in water (luatol) and in oil (trepol), potassium tartrobismuthate in water and oil, sodium tartrobismuthate in water and oil and bismuth trioxid in oil. They have shown, therefore, that the bismuth salts are very much less renotropic and hepatotropic than the soluble and insoluble salts of mercury and practically no histological changes at all were observed in the other organs studied as the adrenals, heart, lungs, spleen and brain.

FIG. 28.—KIDNEY CHANGES PRODUCED BY BISMUTH.
Necrosis and calcification of the spiral tubules of the kidney of a rabbit after 46 daily intravenous injections of sodium and potassium tartrobismuthate (luatol).

(*b*) But in sharp contrast with these relatively mild histological changes produced by intramuscular injections are the rather severe changes produced by *intravenous injections* of the soluble salts and these changes constitute the chief reason for not administering these compounds intravenously except in very small doses. Single lethal or maximum tolerated doses of sodium and potassium tartrobismuthate (luatol), potassium tartrobismuthate and sodium tartrobismuthate produced marked degeneration and necrosis of the epithelium of the convoluted tubules (Fig. 28) with calcareous deposits when the animals survived 3 days or longer. The glomerular loops were frequently blocked by agglutinated erythrocytes, the endothelial nuclei of the tufts were pyknotic and considerable albuminous precipitate occupied the capsular spaces. The liver lesions were generally less extensive but sometimes severe (Fig. 29) and consisted of small areas of focal necrosis, some of which were invaded by leukocytic and monocytic phagocytes, the liver cells in general being

granular and containing numerous hydropic and fatty vacuoles. In the other organs studied (brain, heart, lungs, adrenals and spleen) the changes were inconspicuous and consisted chiefly of capillary emboli of agglutinated erythrocytes and inconspicuous cloudy swelling.

Multiple daily intravenous injections of all three compounds for about 40 injections and totalling approximately 0.117 to 0.149 grams per kilo, produced even more severe changes and particularly of the kidneys and liver. In the former the epithelium of the convoluted tubes was necrotic, desquammated and heavily infiltrated with calcium salts. The inner third of the cortex and that portion of the boundary zone which contains the spiral tubules were particularly involved. The glomerular changes consisted of occlusion of some of the loops by hyalinized erythrocytes and a moderate amount of albuminous precipitate was present in the capsular spaces. In the liver there were occasional areas of necrosis and in

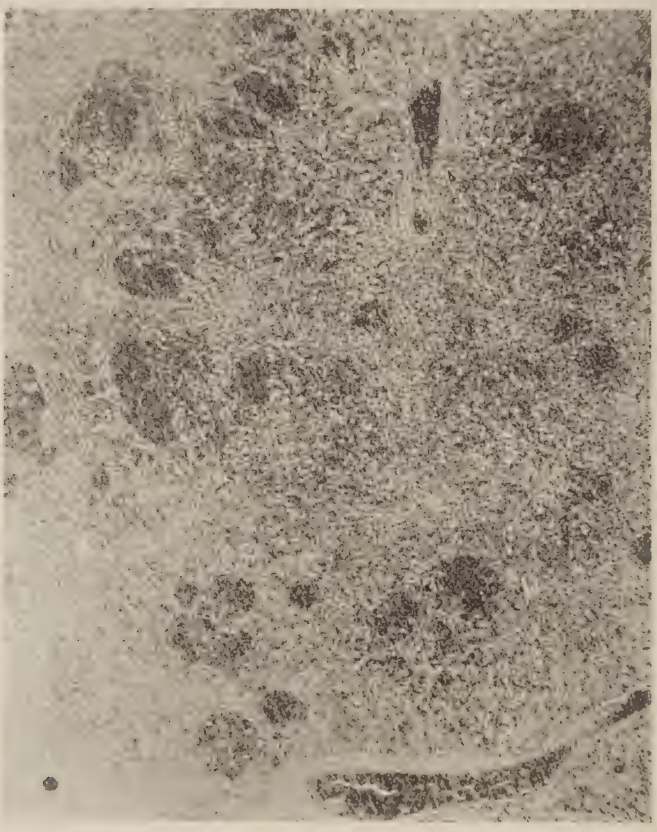

FIG. 29.—LIVER CHANGES PRODUCED BY BISMUTH.

Hemorrhagic and necrotic foci of the liver of a rabbit twenty-four hours after the intravenous injection of 0.060 gm. per kilo of sodium tartrobismuthate.

some instances a few of the bile ducts and portal canals had undergone complete necrosis. The liver cells in general contained an increased number of albuminous granules, were much vacuolated, and often presented nuclear degenerations. Myers and Corbett[10] in a study of the toxicity of several soluble and insoluble salts, found the toxicity greater for the soluble compounds, although the rate of absorption was found to be quite variable for all. In their animals emaciation was one of the first symptoms and histologically, destructive changes were found in the kidneys and liver along with discoloration of the intestinal mucosa, when large doses were administered.

Clinical Significance of the Chemopathological Tissue Changes Produced by Bismuth.—These investigations have shown, therefore, three important

facts, namely, (1) That intramuscular injections of bismuth are very much less toxic than intravenous injections and the lesions produced by the latter are so severe that great caution is required when the soluble salts are given intravenously; (2) that the histological injuries are practically the same for all three of the tartrobismuthates studied either dissolved in water or suspended in olive oil and (3) that bismuth is most toxic for the kidneys, next for the liver and relatively non-toxic for the brain, heart, lungs, adrenals and spleen.

I have not any opportunities for studying human tissues from fatal cases of bismuth poisoning, but believe that essentially similar changes may occur. Furthermore, it must never be overlooked that the pathological changes in human beings produced by *Spirochæta pallida* or other infections may materially reduce tolerance and enhance toxic effects by the drugs. In the treatment of syphilis with bismuth the same care must be exercised in relation to the kidneys as when mercury is administered and especially if arsphenamin or one of its substitutes is administered after a course of bismuth. But unquestionably bismuth is less toxic than the ordinary soluble and insoluble salts of mercury; I would judge that the toxicity is at least 4 or 5 times less and when it is remembered that bismuth is 4 or 5 times more spirocheticidal than mercury it is not difficult to understand that bismuth deserves a place in the therapy of syphilis and especially of late or chronic syphilis.

Symptoms and Lesions of Bismuth Intoxication in Relation to the Treatment of Syphilis.—Investigations with the lower animals have shown, therefore, that the administration of very large amounts of bismuth by intramuscular or intravenous injection may result in the production of tissue changes, particularly in the kidneys and liver, similar to those produced in poisoning with the organic and inorganic compounds of mercury. In my opinion these effects do not necessarily indicate a special tissue affinity of bismuth for renal epithelium and the liver; rather, it would appear that these tissues are injured by reason of their eliminative functions since so much bismuth is excreted through the urine and bile. With ordinary doses of the tartrobismuthates and other compounds of bismuth injected intramuscularly in the treatment of syphilis, the urine is usually free of albumin and erythrocytes indicating that under proper conditions kidney irritation is not ordinarily encountered, but I shall discuss this subject shortly in more detail.

At this writing I do not know of any fatal cases of bismuth poisoning following the intramuscular administration of the tartrobismuthates or other compounds in the treatment of syphilis although sudden deaths have followed intravenous injections as reported by Magnus;[10a] but the lesions described above produced by these compounds in the lower animals were quite similar to those observed in human beings succumbing to bismuth poisoning from the use of bismuth in dusting powders and paste for surgical purposes, as reported by Maher and Baehr,[11] Higgins[12] and others. In other words excessive doses of the tartrobismuthates and other bismuth compounds may produce serious or fatal poisoning in human beings and exactly the same care should be exercised in dosage and intervals of injection as demanded in the administration of mercury to avoid accumulative effects and serious tissue injury.

Etiological and Clinical Classifications of Toxic Reactions.—From the etiological standpoint, toxic reactions are, of course, due primarily to the bismuth administered as the amounts injected intramuscularly or intravenously are too small to warrant serious consideration of toxic reactions

being produced by impurities. But the condition of the patient may contribute in an important manner to the production of toxic reactions, as is true of mercury; for example, if the mouth and gums are in bad condition there is predisposition to siderosis, gingivitis and stomatitis. If the patient is subject to gastro-intestinal disorders, there is predisposition to diarrhea, colic, etc. It is likewise probable that focal infections of the teeth, tonsils and other tissues may predispose to bismuth dermatitis, as in the case of the arsenicals and finally, some individuals appear to possess a natural hypersensitiveness to bismuth so that even very small doses cannot be given These various factors may be summarized, therefore, somewhat as follows:

Etiological classification of reactions
1. Due to the drug.
2. Due to route of administration.
3. Due to predisposing pathological states of individual patients.
4. Due to hypersensitiveness to bismuth.

The toxic reactions that may follow the administration of bismuth are likewise of a varied character. When intravenous injections are given there may occur an acute reaction apparently due to colloidal shock and analogous to similar reactions produced by intravenous injections of other heavy metals; in addition, accidental intravenous injections of suspensions in the course of intramuscular injections may produce symptoms of pulmonary embolism. Siderosis, halitosis, ptyalism, gingivitis, stomatitis and other gastro-intestinal disturbances are most commonly observed; various skin eruptions, renal irritation and provocative Herxheimer reactions are less commonly observed. Local reactions due to muscle irritation and necrosis, may accompany intramuscular injections, the whole subject being briefly classified as follows:

Clinical classification of reactions
1. Acute colloidal shock reaction from intravenous injections.
2. Embolism due to accidental intravenous injections.
3. Nutritional disturbances.
4. Skin eruptions and jaundice.
5. Siderosis, halitosis, ptyalism, stomatitis, and other gastro-intestinal disturbances.
6. Irritation of kidneys.
7. Herxheimer reactions.
8. Local tissue reactions.

General Toxic Effects of Bismuth.—In my experience the earliest symptoms of untoward effects following the intramuscular injection of the tartrobismuthates are general aches in the muscles and joints accompanied by some lassitude and loss of appetite. As a rule, these effects are not severe enough to cause cessation of treatment, and in some instances at least may be due to provocative effect of bismuth on the spirochetes and especially in the case of such lesions as painful lymphatic glands and pain in the heart due to the presence of syphilitic infection of these tissues. At the same time, there is usually some tenderness of the muscles at the site of injection; this symptom varies greatly, however, in different individuals and according to the kind of mixture injected and site of injection.

Acute Colloidal Shock and Embolism Reactions from Intravenous Injections. The intravenous injection of the water-soluble sodium and potassium tartrobismuthate and colloidal bismuth hydroxid, however, have been followed in some instances by acute symptoms of dizziness, pallor, rapid and weak pulse, dyspnea, convulsions and collapse, which are suggestive of a colloidal shock reaction or embolism the result of intravascular agglutination and the production of serum precipitates. Sazerac and Levaditi have strongly

advised, therefore, against the intravenous injection of bismuth on account of these dangers attending its use and if bismuth is administered by this route, the drug should be diluted with at least 10 to 20 c.c. of sterile saline solution and injected very slowly.

In addition to these reactions, the injection of 0.1 to 0.2 grams of the tartrobismuthates suspended in oil into the gluteal muscles has in some exceptional instances been followed in twelve to twenty-four hours by intense aches and pains in the larger bones, joints and muscle groups accompanied by chilliness, fever, a feeling of tightness or compression of the thorax and dyspnea as reported by Shivers.[13] These symptoms are suggestive of embolism due to accidental piercing of a vein, but in the three cases reported by Shivers it was reasonably certain that this accident did not occur and, furthermore, the symptoms did not develop immediately as may be expected if accidental embolism had been produced. The *rheumatoid pains* are especially characteristic and may affect the ribs, spine and particularly the legs as reported by Decrap,[14] Casal[15] and others. They are apt to be continuous, spontaneous and unvarying during the day; they apparently are not influenced by age, sex, stage of syphilis or exercise and are due rather to periosteal intolerance than to Herxheimer reactions.

Chilliness and fever accompanied by rapid pulse and some sweating occurring a few hours after intramuscular injections of bismuth have been described; I have not observed such reactions but it is possible that in exceptional instances the intramuscular injections may produce sufficient irritation and even necrosis of the tissues to engender a protein reaction of this type—a form of non-specific protein reaction produced by the products of tissue necrosis.

Nutritional Disturbances.—In addition, Ducrey[16] has described loss of weight and asthenia as a result of prolonged bismuth treatment in syphilis. I have likewise observed some loss of weight which was attributed to loss of appetite with the appearance of bad taste, foul breath and a blue line of the gums after eight or more injections of 0.2 grams of potassium tartrobismuthate in oil had been given, but as a general rule a course of twelve such injections at intervals of 5 to 7 days were without any general untoward effects.

Bismuth Skin Eruptions.—In the majority of instances no other general untoward effects are observed or complained of following the intramuscular injection of the tartrobismuthates unless stomatitis develops and the injections are continued; under these conditions gastro-intestinal and kidney lesions may be produced and in exceptional instances pruritus and *cutaneous eruptions* may develop, as in the case of exfoliative dermatitis reported by Pinard and Marassi.[17]

Hudelo[18] has reported a case of bismuth stomatitis with cutaneous erythroderma and mentions a case of Gastou and Pontoisseau that received three intravenous injections of colloidal bismuth in five days, which was followed by a confluent and generalized papulo-squamous erythema. Hudelo cites another case reported by Lortat-Jacob and Roberti in which the patient had never received any arsenic and developed an erythema scarlatiniforme from bismuth injections. Levy-Frankel has reported a case of scarlatiniform erythema with purpura after two injections of the iodobismuthate of quinine. Galliot[19] has reported bismuth urticarial eruptions in two women and a scarlatiniform eruption on an infant's buttock on the side of the injection. Lepinay[20] has reported a man that received neo-arsphenamin and grey oil without any reaction and two months later was given one injection of the iodobismuthate of quinin. Large urticarial plaques with pruritis of the

back, fever and headache occurred the next day. Nicolas[21] has reported a bismuth lichenoid eruption and a localized eczema, also a rubeoliform and discreet purpura of the extremities that Fournier and Guénot mention. Lehner[22] has reported a tabetic that developed a toxic erythema with herpes zoster of the right thigh after bismoluol. This patient was also sensitive to other bismuth salts, as shown by intracutaneous tests. Sarateanu[23] has also recorded a case of general erythrodermia from injections of neotrepol and Freudenthal[23a] has recently described three interesting cases presenting local exanthems due to embolism following intragluteal injections of bismuth.

Bismuth Jaundice.—I have never seen a case of jaundice due to injections of the tartrobismuthates and this condition is apparently a very uncommon accident in bismuth therapy of syphilis. Hudelo and Rabut[24] state that it may occur, but rarely, and that bismuth may be given to cases recovered from arsphenamin jaundice without danger of recurrence. As previously stated histological studies have shown that the liver is not involved, except in severe bismuth intoxications from intravenous injections.

Bismuth Siderosis and Halitosis.—Probably the earliest untoward effects produced by intramuscular injections of bismuth tartrobismuthate are local irritation and a slight degree of general muscle aches and some loss of appetite, the development of a bad taste, coated tongue, disagreeable odor of the breath and a blue line (siderosis) along the margins of the gums. Sometimes the *halitosis* or odorous breath precedes the appearance of the blue line, but generally both develop at the same time and forecast the appearance of stomatitis and are associated with it. While the breath is disagreeable and the patient may be conscious of a bad taste in the mouth, fetor is not present as in mercurialism unless a well marked stomatitis is present.

The *blue line* first appears on the gingival margin of the incisor teeth, both anteriorly and posteriorly, and may later spread if treatment is continued. It is indistinguishable from that seen in plumbism and develops in the majority of patients after ten or more intramuscular injections of the tartrobismuthates in dose of 0.1 to 0.2 grams in oil every four or five days. Some individuals are more susceptible than the average and may show a blue line after three or four injections. According to Azoulay[25] histological examination of the gums impregnated with bismuth shows that the drug is eliminated only on the dental side of the gums. Milian and Perion[26] state that the pigmentation is due to the presence of bismuth particles in the papillæ of the dermis and especially in the endothelial cells of the capillaries where they are carried by the blood. According to Meyer and Steinfield sulphids of bismuth are produced and it is believed that the deposits may be so heavy as to cause obliteration of the vessels and consequent necrosis with the production of ulcers.

Blue spots may also appear under the tongue and in the buccal mucosa of the cheeks. Evidently this pigmentation of both gums and buccal mucosa is due primarily to depositions of bismuth, as shown by Klare[26a] and others, brought to these tissues for elimination and it is entirely likely that similar changes occur in the mucosa of some parts of the intestines and especially of the first portion of the colon, responsible for the constipation and anorexia sometimes accompanying this condition.

As a general rule the blue line and spots tend to disappear rapidly after cessation of treatment; Milian states that they may disappear in five to fifteen days and Klauder[27] has observed their disappearance in a month after stopping treatment.

Bismuth Gingivitis, Stomatitis, Ptyalism and Other Gastro-intestinal Disturbances.—If the administration of bismuth is continued in full dosage after

the development of a blue line of the gums, gingivitis may be produced. Ordinarily this is a mild localized inflammation which tends to disappear in a few weeks when treatment is interrupted, but severe gingivitis and stomatitis may be produced as reported by Chatelein and Hude[27a] and others. Changes of this kind are exceptional among subjects whose gums are healthy and sound and who are careful in maintaining a proper hygienic condition of their mouths. On the other hand, among individuals with soft, spongy, bleeding and tender gums with tartar deposits, bismuth gingivitis is apt to be more severe and generalized owing to the fact that bacteria and spirochetes add to the irritation and inflammatory changes.

The gingivitis is apparently caused by the depositions of bismuth in the walls of the capillaries causing partial obliteration with resulting congestion and loss of nutrition and the production of minute ulcers. These ulcers develop, therefore, in the areas of most intense pigmentation and are surrounded by greyish-blue borders of bismuth deposits. Bacteria then add to the inflammatory changes and Azoulay believes that the fusiform bacillus and spirochete of Vincent are especially likely to produce severe ulceration under these circumstances with the development of greyish exudates and false membranes. In such cases the submaxillary and other neighboring glands become swollen and painful by reason of this secondary bacterial infection. In a few cases of severe bismuth stomatitis which I have had the opportunity of examining bacteriologically, enormous numbers of fusiform bacilli and spirochetes were found as described by Azoulay, along with other various other bacteria.

In exceptional cases gingivitis is followed or accompanied by generalized stomatitis but only among patients whose gums and teeth are in very bad condition, or who are subject to gastro-intestinal disturbances with frequent shallow irregular ulcers on the sides of the tongue or of the buccal mucosa. The symptoms resemble those of mercurial stomatitis with foul breath, painful mastication, anorexia and malaise but the ulcers tend to heal more rapidly after bismuth injections are stopped.

Ptyalism is unusual in bismuth gingivitis, occurs only in well-marked cases and even then is moderate and not nearly as severe as in mercurial gingivitis and stomatitis. While ptyalism is usually an early sign of impending mercurial gingivitis this is not the case in bismuth gingivitis and the physician must not depend upon the development of this sign as the first indication of over-dosage; rather the gums should be inspected at least once every week for the blue line as the earliest sign of untoward effects.

Since the production of gingivitis and stomatitis is due to the metal bismuth itself, one naturally expects that these effects vary according to the bismuth content, solubility and dosage of the compound injected. Injections every 2 or 3 days of any bismuth preparation is more apt to produce discoloration and inflammation than when given at intervals of 5 to 7 days, owing to the bringing of more bismuth to the mouth for elimination than the tissues can excrete. A single dose of 0.5 grams of the tartrobismuthates is almost sure to produce gingivitis according to Levaditi, Milian, Azoulay and others whereas in doses of 0.1 to 0.2 grams at intervals of 5 days as much as twelve to fifteen injections totalling 2 to 3 grams may be given without bad effects. With these doses the blue line may be produced but severe gingivitis is exceptional, as shown by the experience of Milian and Perin.[28] Apparently, the balance of the gastro-intestinal tract does not suffer nearly as much as the mouth during the elimination of bismuth. Milian[29] and Azoulay[30] have reported cases of severe enteritis and *ulcerative colitis* but these are quite rare.

The tongue may be streaked and the patient may complain of nausea, anorexia and constipation but ordinarily no other effects are noticed and even these do not usually develop. No doubt bismuth is also eliminated through the intestinal mucosa and especially that of the colon in much the same manner as in the mouth and very probably the mucosa may be discolored, but severe inflammation and ulceration does not develop during the treatment of syphilis with bismuth due to the small doses ordinarily injected at intervals of 3 to 7 days and more especially because the intestinal mucosa is ordinarily in a healthy condition without predisposing bacterial injuries as are so common in the mouths of adult individuals. For this reason I believe that preexisting gingivitis or pyorrhea is the most important single factor predisposing to the development of bismuth gingivitis and stomatitis during the treatment of syphilis with bismuth and mercury.

Effect of Bismuth upon the Kidneys.—While as previously stated bismuth may produce tubular nephritis when administered to the lower animals (rabbits and rats) in single massive doses, yet bismuth is not nearly as likely to produce renal irritation as mercury. *Polyuria* may be observed in exceptional instances as stated by Fournier and Guegnot,[31] but this sign of renal irritation is not nearly as common as follows the administration of mercury; in my experience patients treated with intramuscular injections of 0.1 to 0.2 grams of potassium tartrobismuthate every 5 to 7 days rarely observe an increase of urine and the measure of a series of 24 hour outputs has not usually shown an increase above the normal fluctuations observed before bismuth treatment was begun.

Albuminuria has been reported by Simon and Leoy-Buig,[32] Fischer[32a] and others due to the administration of bismuth in the treatment of syphilis, but this sign of renal irritation is observed only in exceptional cases and Azoulay states that neither albuminuria nor cylindruria develop after the intramuscular administration of "trepol" (a suspension of sodium and potassium tartrobismuthate in oil). Hudelo and Rabut[33] state that they have observed albuminuria only 4 times in 10,000 injections of bismuth. Klauder observed only a few cases in his series treated with the tartrobismuthates and other bismuth compounds; Shivers likewise reports that in his series albuminuria did not occur but that on the contrary, several cases of presumably syphilitic albuminuria were greatly benefited by injections of bismuth. Blum[34] is of the opinion that albuminuria is only likely to develop in cases of stomatitis and that it may be due in part to the associated bacterial infection or be an expression of syphilitic irritation requiring a continuation rather than a cessation of treatment.

Rathery and Richard[35] report that large doses of sodium and potassium tartrobismuthate in oily suspension administered to dogs produced no marked lesions, even in cases where a nephritis had previously existed. However, cylindruria was aggravated in cases where it was present before the injections, showing that the kidneys were affected by the administration of bismuth. The urine and *renal function* of 16 patients undergoing tartrobismuthate treatment were also studied. In some patients a slight polyuria was noted, but this was far from constant and the previous condition of renal function did not seem to influence its appearance. When albuminuria was absent before the injections it did not appear thereafter. Two of the patients had a normal Ambard's quotient, and azotemia and phenolphthalein excretion before and after treatment. Of the other 14 cases who had definite signs of renal deficiency before treatment, 6 were slightly aggravated in this respect, 3 showed no changes and 5 were definitely improved. The favorable effects

observed in this last group were perhaps due to the fact that in those cases there were curable syphilitic lesions in the kidneys. The authors conclude that, while a deficient renal function does not contraindicate of themselves the administration of bismuth in syphilis, such treatment must be carefully supervised.

Kollert, Strasser and Rosner[36] observed slight albuminuria only rarely during the treatment of 39 syphilitics with trepol in dose of 0.2 to 0.3 grams at not less than 4 day intervals until 3.0 grams had been injected. On the other hand, in 27 cases an unusual sediment of small, polygonal finely granular epithelial cells was noted. In 4 cases a few erythrocytes were found but in none of the patients were there any symptoms of nephritis, such as edema. The authors also observed slight diuretic effects which were not however, as marked as after the administration of mercury, and they state that examinations of the urine for albumin alone may be insufficient for detecting irritation of the kidneys.

It would appear, therefore, that while large single doses of bismuth may produce renal irritation and stomatitis, multiple small doses at intervals of 3 to 7 days are not at all likely to produce these effects, since time is allowed for excretion before excessively large amounts accumulate in these organs. In my experience the intramuscular injection of a series of twelve to fifteen doses of 0.1 to 0.2 grams each of the tartrobismuthates at intervals of 5 to 7 days has rarely produced albuminuria or other signs of renal irritation and in several cases albuminuria following the injection of mercury has gradually disappeared under this kind of bismuth therapy. I believe, however, that it is well for the urine to be examined microscopically and for albumin routinely every two weeks at least during courses of bismuth injections and especially if there are evidences of gingival siderosis or stomatitis.

Provocative or Herxheimer Reaction after the Administration of Bismuth in Syphilis.—Klauder has observed that in syphilitic orchitis of rabbits the intramuscular injection of potassium tartrobismuthate in dose of 0.05 gram per kilogram was sometimes followed in forty-eight hours by increased swelling of the testicles and other signs of renewed spirochetic activity or the effects of toxic substances derived from the organism as a result of the injection.

These local reactions may be interpreted, therefore, as a provocative or Herxheimer reaction. In syphilitic human subjects I have occasionally observed the occurrence of pain and tenderness of enlarged lymph glands and tertiary skin lesions after one to three intramuscular injections of 0.2 gram each of potassium tartrobismuthate; also vague aches and pains in the heart muscle and other parts of the body, which may have been due to similar provocative effects. In some cases of latent tertiary syphilis the complement-fixation reactions have become stronger, and these effects are to be likewise interpreted as due to provocative stimulation or a serological Herxheimer reaction even though there were no clinical evidences of exacerbation of the disease.

Bismuth, therefore, may produce mild Herxheimer reactions in syphilis, that is, cause an exacerbation of the disease or of a particular lesion or lesions either by stimulating spirochetes into renewed activity or releasing from them by lysis toxic substances capable of exciting inflammatory reactions; these effects, however, are not nearly as common as after the intravenous injection of arsphenamn, neoarsphenamin or mercury because absorption is so much slower by intramuscular injection. Indeed, exacerbations of this kind are decidedly uncommon in the treatment of syphilis with intramuscular injections of 0.1 to 0.2 grams of potassium tartrobismuthate in oil and I do not

hesitate to administer these amounts even in neurosyphilis when the occurrence of a "neurorecidiv" reaction is to be feared and carefully avoided.

Local Reactions Produced by Intramuscular Injections of Bismuth.—The degree of local inflammatory reaction including pain produced by the intramuscular injection of bismuth varies considerably according to the nature of the compound and the vehicle employed. For example, sodium and potassium tartrobismuthate dissolved in water (luatol) is ordinarily so painful that a series of injections cannot be given, whereas when suspended in a vegetable oil (trepol) it is well tolerated and according to Klauder, suspensions of bismuth trioxid in olive oil are less painful than sodium and potassium tartrobismuthate in the same oil.

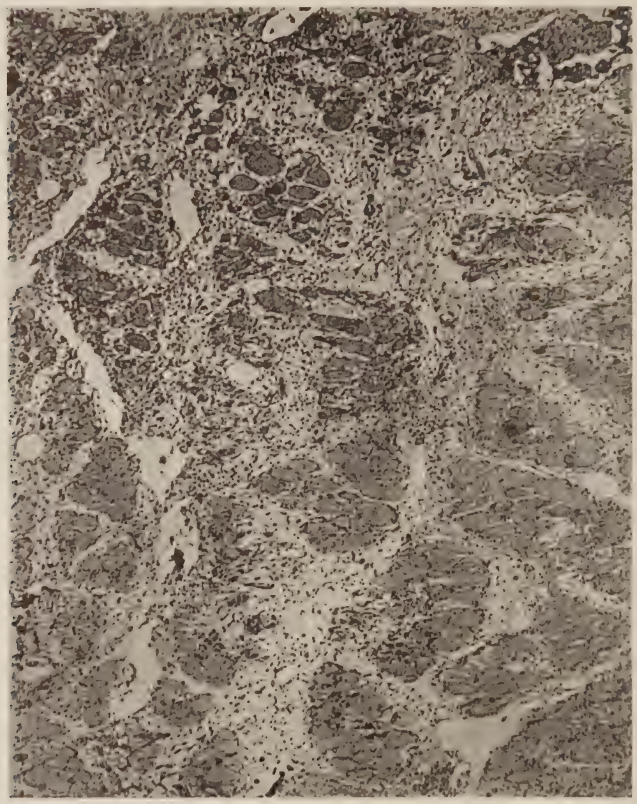

FIG. 30.—LOCAL REACTION PRODUCED BY THE INTRAMUSCULAR INJECTION OF BISMUTH IN OIL.

Injection of 0.025 gm. of potassium tartrobismuthate in 0.5 c.c. of almond oil (six days).

On the other hand, the degree of pain and discomfort following the intramuscular injection of any preparation varies considerably among different individuals even when extraordinary precautions are taken to inject each with the same dose and in exactly the same manner; furthermore, among individuals receiving a series of injections, the pain and discomfort varies even though the same dose is given, although not exactly at the same site. In the majority of instances the intramuscular injection of 0.2 grams of potassium tartrobismuthate suspended in 2 c.c. of sterile olive oil with the addition of 10 minims of a 2 per cent. solution of butyn, has produced no

immediate discomfort, but an area of tenderness and some pain at night when lying in bed, and disturbance of sleep. The injected area is ordinarily slightly tender for several days but may prove so annoying in nervous "high strung" patients as to constitute an obstacle to repeated injections. On the other hand this preparation produces somewhat less pain and discomfort and is better borne than grey oil, suspensions of salicylate of mercury or calomel in oil.

Histological studies of muscles excised about five days after injections of the tartrobismuthates in rats have shown that these salts produce varying degrees of necrosis of those muscle cells in direct contact with the deposited drugs accompanied by well marked degrees of leukocytic infiltration. The effects produced by potassium tartrobismuthate, sodium tartrobismuthate and the quartenary salt of sodium and potassium tartrobismuthate are quite similar in kind and degree, but the reactions produced by solutions of these in sterile water are almost always more severe than similar doses in sterile almond oil (Fig. 30). The water solutions produce a smaller, but more intense reaction which may progress to the stage of liquefaction necrosis; the oily suspensions produce a more diffuse reaction in which there is less necrosis of muscle but in which the neighboring muscle is more broadly infiltrated with polymorphonuclear leukocytes.

Why the same preparation administered in exactly the same dose, and at least approximately if not exactly in the same area, should excite a greater degree of local reaction in some individuals than in the majority, is not known; however, the reactivity of the skin of different persons to intradermal injections of various substances is known to vary in that pustular reactions may develop in some and almost no reaction at all in others injected with the same substance in the same dose and in the same manner. Individuals taking the iodids are especially likely to develop pustular reactions following intradermal injections and I believe that the same mechanism may influence the reactions following intramuscular injections. In other words the kind and degree of inflammatory reaction that may be excited in the muscles varies, not only according to the direct irritating properties of the medicament injected but also according to the degree of sensitiveness of the individual to any irritant; for this reason some individuals react excessively with dense collections of leukocytes and the tendency for pus production, while others react scarcely at all so that the matter of pain following intramuscular injections is not altogether a matter of a general "high strung" or nervous disposition and intolerance for pain.

In this connection, it is interesting to note that intramuscular injections of the tartrobismuthates to rats, after the daily administration of potassium iodid by stomach tube for five days, have frequently produced more severe local histological changes than in control rats receiving only the intramuscular injections. In the iodid rats the local lesions were larger and more pustular. However, this influence of potassium iodid is not demonstrable clinically; I have many times given a series of intramuscular injections of potassium tartrobismuthate in dose of 0.2 grams in 2 c.c. of sterile almond oil every five days while patients were taking internally from 15 to 60 grams of potassium iodid daily without noting that the local reactions were in any manner larger or more painful than in cases to whom the iodids were not being administered.

As previously stated, water solutions of the tartrobismuthates are more painful and produce more severe histological changes than suspensions in sterile almond or olive oils. This is readily understood, because the oil is bland and soothing and prevents the sudden contact of the bismuth compound with the tissues; furthermore, absorption of the oil is delayed since

soaps are produced by contact of the vegetable oil with the alkaline tissue juices. Mineral oils, as paraffin oil, do not saponify and greatly reduce the rapidity of absorption without, however, appreciably reducing the pain or tenderness.

Nodules in the tissues following intramuscular injections are purely a matter of inflammatory reactions and rapidity of absorption of drug and exudates. In my experience the tartrobismuthates in dose of 0.1 to 0.2 grams suspended in 1 to 2 c.c. of sterile olive or almond oils produce less nodulation than when dissolved in 1 to 2 c.c. of water or suspended in 1 to 2 c.c. of sterile paraffin oil.

In any event the addition of phenol and a local anesthetic as butyn or anesthesin to either watery solutions or oily suspensions of the tartrobismuthates greatly reduces the immediate pain and discomfort of injections and should not be omitted; the formulas proving especially useful are given in Chapter LXIV.

Mechanism of the Toxicological Action of Bismuth in the Treatment of Syphilis.—The toxicity of the various organic and inorganic compounds of bismuth advocated and employed in the treatment of syphilis apparently depends upon the dissociated ions of bismuth itself in just the same manner as the toxicity of the mercurial compounds depends upon the dissociated ions of mercury. When the soluble salts are injected intravenously, toxic effects may be due to immediate or almost immediate intravascular changes similar to the immediate reactions sometimes following the intravenous injection of the organic arsenical compounds and particularly arsphenamin; when injected intramuscularly, these immediate reactions are not likely to occur as absorption is so slow that the intravascular reactions do not result or are of a minor grade and without symptoms.

In general terms it would appear that the toxicity of various bismuth preparations bears a relation to the amount of bismuth contained and this would be true if the compounds were equally soluble in the tissue fluids and if the bismuth ions were dissociated with equal rapidity from the different compounds. While the toxicity of the organic and inorganic mercury compounds bears a relation to the amount of mercury contained in each yet toxicity has an even closer relation to the degree of solubility of the compounds and more particularly to the degree of dissociation of the mercury ions; for example, a full complex compound may contain more mercury than a half or pseudocomplex compound and yet prove less toxic (and likewise less therapeutic) than the latter because the mercury is so firmly bound that the compound may be absorbed and eliminated in large part unchanged. I believe that exactly the same principles govern the toxicity of bismuth compounds for both the host (organotropism) and the parasites (parasitropism).

The toxicological action of bismuth is similar to that of mercury in other respects as well. For example bismuth acts upon the epithelium of the uriniferous tubules of the kidneys in exactly the same manner producing albuminous degeneration and final liquefaction necrosis; the deposition of calcium in the degenerated cells likewise occurs as in mercurial intoxication but not as extensively. Similar effects are produced in the liver, in the muscles at the site of injection and other organs; the degree of reaction in all organs is, however, less than occurs with mercury so that it appears that the toxicity of bismuth ions is less for the body cells than mercury ions although at least five times more toxic for *Spirochæta pallida* than the latter.

Toxicity of bismuth, of course, bears a relation to the condition of the eliminative organs. This is seen particularly well in the mouth since individuals with Rigg's disease or gingivitis (pyorrhea alveolaris) are cer-

tainly more prone to siderosis and stomatitis than those whose gums are sound and healthy. Such individuals may show no signs of toxicity elsewhere although albuminuria is more likely to develop if stomatitis is present. Apparently the buccal mucosa, kidneys and intestinal mucosa are able to eliminate promptly and without apparent injury the bismuth dissociated from 2 to 3 grams of the tartrobismuthates given intramuscularly in doses of 0.1 to 0.2 gram over 10 to 12 weeks of time, provided these organs are sound and healthy; if these tissues are inflammed, however, and particularly the gums, the bismuth is precipitated at the points of elimination and converted into solid particles producing siderosis (blue line) and ultimately ulceration, if the bismuth accumulates in the tissues and capillary walls to a sufficient degree to occlude the vessels.

The mechanism of production of siderosis, stomatitis and allied lesions is, therefore, fairly well established, but it is more difficult to explain the mechanism of the bone and muscle pains, particularly of the legs, thoracic pains and the skin lesions. It is probable that the former are due to neuritis in a manner analogous to arsenical and mercurial neuritis; the latter may be due to irritation produced by the elimination of bismuth through the excretory channels of the skin, to a state of hypersensitiveness to bismuth or the result of bacterial anaphylaxis from foci of infection activated by the depressive and toxic effects of the drug.

Mechanism of Toxic Reactions Following Intravenous Injections.—Just why the water soluble tartrobismuthates and other soluble compounds of bismuth injected intravenously should prove so toxic as to render unsafe and unwise this route of administration is not clear. Mercury for example, is certainly more toxic than bismuth by intravenous as well as intramuscular routes of administration since the maximum tolerated dose of the bichlorid is about 0.006 gram per kilo while the soluble tartrobismuthates are tolerated in doses of 0.020 to 0.030 gram per kilo. Furthermore, the bichlorid and other soluble compounds of mercury can be given intravenously with safety if well diluted and slowly injected although repeated injections may produce plebitis is some instances. I believe that the dangers of intravenous injections of the tartrobismuthates have been overemphasized by Levaditi and others although there does not appear to be any important reason why the intravenous route is the one of choice since absorption is so prompt from the muscles, unless it is for the treatment of acute syphilis when the organic arsenical compounds as arsphenamin or neoarsphenamin cannot be given because of hypersensitiveness.

The symptoms produced as dyspnea, cough, thoracic oppression, etc., and some of the lesions found in the lower animals as emboli of agglutinated erythrocytes in the capillaries of the lungs, liver and kidneys, folllowing the intravenous injection of large amounts of the tartrobismuthates, suggest that bismuth may produce masses of precipitated plasma proteins and agglutinated erythrocytes when injected intravenously in much the same manner as these may be produced by arsphenamin. My experiments have shown that sodium tartrobismuthate and potassium tartrobismuthate may agglutinate *washed* human erythrocytes in final dilutions as high as 1:30,000 to 1:50,000 in an exposure of one hour although hemolysis is produced only in final dilutions of 1:100 to 1:200. Similar results were observed with washed sheep corpuscles, but when the tests were conducted with *unwashed* human and sheep blood it was found that agglutination and hemolysis did not occur even with final dilutions as low as 1:200. In other words, the presence of serum protects erythrocytes against agglutination and hemolysis by bismuth *in vitro* just as serum protects erythrocytes against agglutination and hemolysis

in vitro by arsphenamin and mercury, as described in preceding chapters. These results, of course, greatly reduce the significance to be attached to the agglutinating and hemolyzing properties of bismuth as factors responsible for immediate reactions when the soluble salts are injected intravenously. Furthermore, sodium tartrobismuthate and potassium tartrobismuthate added to serum *in vitro* produce insoluble albuminates much more slowly than mercuric chlorid, and I doubt if precipitation of plasma proteins occurs to sufficient extent intravascularly to be of primary importance from the toxicological standpoint.

Doubtless, however, intravenous injection of the soluble tartrobismuthates and other compounds of bismuth may produce the same kind of obscure colloidal and other changes in the plasma as may follow the intravenous injection of arsphenamin and mercury and it is barely possible that these effects are due primarily to alkali and other substances aside from the bismuth itself, but the exact mechanism of these immediate reactions is just as obscure as is true of the early or immediate arsphenamin reactions and I believe that they could be avoided or greatly minimized by the same precautions observed in the administration of arsphenamin, namely, to inject the compound slowly and after being well diluted with saline solution.

Prophylaxis of Toxic Reactions by Bismuth.—All that has been written in Chapter LIII regarding the prophylaxis of toxic reactions by mercury applies equally well to bismuth. Special attention should be given the condition of the teeth and gums and throughout the administration of bismuth the patient should be carefully instructed, if necessary, regarding the proper cleansing of the teeth and gums and the hygiene of the mouth. Precautions of this kind go far in preventing stomatitis, although the danger of its production is not as great as when mercury is administered.

Constipation and dietary errors should be corrected as likewise other gastro-intestinal disorders as may be amenable to improvement or removal.

Renal irritation and albuminuria do not necessarily contraindicate the administration of bismuth, but it is well to carefully examine the urine once every 2 weeks at least to detect or guard against the production of bismuth injury of these organs.

If a soluble salt is administered by intravenous injection, the dose should be well diluted and slowly injected to prevent or minimize the colloidal shock reaction that may follow. When intramuscular injections are given, due precautions should be taken against accidental intravenous injection.

Local reactions are minimized by giving them strictly intramuscularly or epifascially as described in Chapter LXIV; subcutaneous injections are apt to be more painful and result in nodosities. The immediate pain is well controlled by incorporating an anesthetic.

Treatment of Bismuth Toxic Reactions in Syphilis.—Insofar as bismuth poisoning is concerned in relation to its administration in the treatment of syphilis, only stomatitis requires much consideration.

For the acute early reactions that may follow intravenous and rarely intramuscular injections, symptomatic treatment only may be given, as there is nothing in the form of specific medication since so little is known of the exact mechanism of these reactions. Acute bismuth poisoning due to over dose is not likely to occur since bismuth is injected by the physician and the opportunities for error on the part of the patient are not nearly as great as in the case of mercury when toxic amounts may be swallowed. In case of acute poisoning due to the accidental administration of an excessive dose, I believe that the most dangerous lesion would be acute tubular nephritis with symptoms similar to acute bichlorid of mercury poisoning and requiring exactly

the same kind of treatment including the intravenous injection of sodium thiosulphate.

As a general rule, the vague muscular pains, malaise and anorexia following the first few doses disappear as treatment is continued; if, however, these symptoms persist with increasing anemia, loss of weight and with the appearance of siderosis of the gums, the dose injected should be reduced or the administration stopped.

When bismuth is administered, the gums should be examined at every visit of the patient and it is well not to begin the injections if severe gingivitis is present until the gums have received appropriate preliminary treatment and especially removal of tartar deposits with some degree of healing as well as the extraction of decayed roots and the filling of carious teeth.

The appearance of a slight blue line about the incisor teeth does not necessarily mean that treatment must cease if the gums are otherwise healthy. It is my practice to routinely inject 0.2 gram of potassium tartrobismuthate intramuscularly every 5 days and to reduce to 0.1 gram as the dose upon the first appearance of siderosis. Under these conditions a full course amounting to a total of about 2 grams can usually be given in 8 to 12 weeks.

If stomatitis develops and particularly if ulceration occurs with halitosis, the injections should cease at once and appropriate treatment applied to the gums. Bacteriological examination usually discloses the presence of enormous numbers of spirochetes with numerous fusiform bacilli and when such is the case, I believe that local applications of arsphenamin or neoarsphenamin yield best results, as described in Chapter XXXI. Palazzi[37] states that the local application of a 100 per cent. solution of methylene blue is efficacious and that arsphenamin or neoarsphneamin injected intravenously has an undoubted curative influence upon these spirochetic infections. Potassium chlorate mouth washes are also helpful.

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CHAPTER LVI

PHARMACOLOGY OF THE IODIDS IN RELATION TO THE TREATMENT OF SYPHILIS AND OTHER DISEASES

Since 1836, when potassium iodid was first advocated by Wallace, of Dublin, for the treatment of syphilis, this remedy has remained an almost undisputed agent in the therapeutics of this disease and especially for the treatment of the tertiary lesions, as so early shown and emphasized by Ricord. Even to this day, however, the exact mechanism of its wonderful curative effects is not definitely known, and we can only state that evidence shows that, in the amounts ordinarily administered, it possesses but feeble spirocheticidal effects. But the wonderful manner with which it brings about the absorption of inflammatory exudates and fibrous tissue, not only in syphilis but in other infections as actimomycosis, sporotrichosis, tuberculosis, etc., renders the iodids of great interest and importance in the chemotherapy of these infections, as I shall discuss shortly with more detail.

Chemistry of the Iodids in Relation to the Treatment of Disease.—Naturally, with a remedy so valuable, a very large number of new inorganic and organic compounds of iodin and iodid have been described and advocated for the treatment of syphilis and other diseases. But we are not at all interested in the iodin compounds, insofar as the chemotherapy of syphilis and other infections are concerned, because it is well established that in these it is the iodid ion and not the iodin ion that is of importance. The inorganic iodids, however, like potassium iodid and sodium iodid are rapidly eliminated and numerous attempts have been made to produce organic compounds of slower dissociation and elimination of the iodid ion. For this purpose a very large number of iodo-albuminates, -peptonates, -tannates, etc., have been prepared, but, as shown by McLean[1] and others, these are so rapidly decomposed that they behave and are excreted practically like the inorganic iodids and have no advantage over either sodium or potassium iodid. However, combinations of iodids with oil (*iodipin*) and fatty acids (*sajodin*), as well as *iodostarin* and *lipoiodin*, are absorbed, decomposed and excreted more slowly. McLean found that they are largely retained in the lipoid-soluble fraction of the cells and, therefore, likely to penetrate the nervous system more freely than the inorganic iodids; Jacoby,[2] however, states that iodin is not stored in tissue fat when iodized fatty acids are administered as such by ingestion, but only when given in the form of their calcium salt.

But for all practical purposes, potassium iodid and sodium iodid are quite satisfactory for the treatment of disease, although a good working knowledge of their absorption, distribution and retention in the tissues, and especially their elimination, is required in order that they may be properly administered in such manner as may maintain the maximum curative concentration in the tissues and fluids for sufficient periods of time. Attempts to reduce the toxicity of the iodids are praiseworthy, but hardly worth while, since both salts and particularly sodium iodid are of remarkable low toxicity for both human beings and the lower animals, although some individuals possess a marked hypersensitiveness to the iodid ion, regardless of the chemical nature, organic or inorganic, of the compound by which it is introduced into the body.

Iodid compounds of mercury, lead, etc., have not proved useful from the standpoint of iodid therapy, because the high toxicity of the metal prevents the administration of sufficient amounts to give well-defined iodid effects. But if the iodid ion could be introduced into the arsphenamin or neoarsphenamin molecule without greatly increasing toxicity, I believe that a distinct achievement and advance in the chemotherapy of syphilis, especially in its tertiary stages, would result. And if success attends present and future efforts to evolve a chemotherapy for tuberculosis, actinomycosis, sporotrichosis, etc., I believe that the introduction of an iodid ion will greatly aid in localizing the parasiticidal agent of the compound in the diseased tissues and aid in opening paths of access to the infectious agents.

For all practical purposes the two inorganic salts, potassium iodid and sodium iodid fulfill the requirements for the treatment of infectious diseases; iodin or compounds liberating free iodin are of no particular interest in the treatment of syphilis, actinomycosis, sporotrichosis, etc.

Of these two salts, sodium iodid is much less toxic than potassium iodid—at least six or seven times less toxic, as will be shortly described—and is to be preferred to potassium iodid for intravenous injection. The difference in toxicity is due to the dissociation and higher toxicity of potassium and the more rapid excretion of sodium iodid; the iodid ion appears to be of the same toxicity in both. Ammonium iodid is said to be more liable to produce the skin eruptions and gastrointestinal disturbances. Occasionally these iodids cannot be administered by ingestion in adequate doses to some patients by reason of gastric and other disturbances; in these I have sometimes found pepto-iodin, prepared by Raiziss, better borne, as likewise, lipoiodin and other organic compounds.

Absorption of the Iodids in Relation to Methods of Administration.—The iodids are rapidly absorbed from mucous membranes, so that, with oral administration, the absorption occurs principally from the stomach, the peak of highest concentration of iodid and iodin in the blood being reached about one hour after the ingestion of 15 to 45 grains and about two hours after the ingestion of 300 grains. Absorption through the skin, however, is so slight as to be therapeutically worthless. Absorption also occurs from the intestine including the rectum.

Fournier states that, in 1867, examinations of the urine from a case of ectopic bladder with the ureters opening about three inches above the pubis, so that the urine could be collected from the moment when it first began to drop, showed the presence of iodid after the following intervals:

After ingestion by the stomach	14 or 15 minutes.
After placing on the tongue	14½ minutes.
After subcutaneous injection	20½ minutes.
After injection into the rectum	23 minutes.

Hanzlik,[3] in 1912, found that the absorption of sodium iodid solutions placed in ligated loops of small intestine, was nearly equal in all parts; absorption from the stomach and colon was somewhat less. The rapidity of absorption was not materially influenced by the extent of surface, or by the concentration of the solution. The absorption was very rapid at first, but slower after absorption of the first one-half to three-quarters of the amount injected due to local changes in the epithelium. Sodium iodid is probably absorbed somewhat more rapidly than potassium iodid; at least it is excreted more rapidly, as will be shortly discussed.

It is sometimes recommended to administer the iodids by ingestion with fats in the form of milk and cream on the theory that the lipoids hasten absorption. The observations of Osborne,[4] however, have not substantiated these claims, as he observed no difference in the rate of absorption of potassium and sodium iodids due to fats in ordinary meals, as compared with the empty stomach. *Patients noted less gastric irritation when the drugs were taken on an empty stomach one-half hour before meals.* Sodium iodid was better tolerated, both by ingestion and intravenous injection, than potassium iodid.

Within recent years sodium iodid has been recommended for intravenous injection, but, owing to the rapid absorption from the stomach and intestines, this route does not appear to be indicated except in exceptional cases of syphilis, sporotrichosis, etc., when sufficient amounts cannot be given by ingestion owing to gastro-intestinal disturbances or when a very high concentration in the blood for a short time is required for extra rapid effects. Osborne, for example, has found that, after the intravenous injection of 10 gm. of either sodium or potassium iodid to adults, the iodin content was 0.043 gm. for 100 c.c. of blood serum at the end of one hour, 0.0247 gm. at the end of four hours, 0.0071 at the end of twenty-four hours and only a trace by the end of the fourth day. However, the oral administration of 3 to 5 gm. (45 to 75 grains) three times a day is equal to a single dose of 10 gm. (150 grains) intravenously, and has the added advantage of producing a uniformly sustained concentration of iodid and iodin during the day. The giving of large doses by vein produces a very much higher concentration for a much shorter period. Oral administration creates a moderate but prolonged concentration in the blood and tissues; intravenous administration of large doses creates peaks of high concentration followed by rapid declines.

It would appear, therefore, that, for routine treatment, the iodids should be administered by ingestion and intravenous injections employed only in exceptional cases. As stated by Osborne, it seems that unless intravenous injections are used as adjuncts to oral administration, there is no special advantage in giving small doses intravenously. In the treatment of neurosyphilis, however, intravenous injections of 10 to 20 gms. of sodium iodid (100 to 200 c.c. of 10 per cent. solutions) may be advantageous, since these are followed by a marked increase of iodid in the cerebrospinal fluid and possibly also in the syphilized portions of the brain and cord, much higher than can be secured by oral or rectal administrations, as shown by Osborne and to which further reference will be shortly made.

In exceptional cases of gastric intolerance, the iodids may be administered by rectum, at least for a period of a week or two at a time. Absorption is quite rapid; according to some pharmacologists, better than from the stomach, and it is possible to administer solutions by this route. According to my experience suppositories are less well borne, apparently because of irritation due to concentration of the drug. Osborne has observed, however, that when 3 to 10 gms. of potassium iodid were given two patients by drop

proctoclysis per day, only 16.9 to 43.3 per cent. of the iodin was excreted in the urine. Increasing the dose above 10 gm. daily did not produce a corresponding increase in absorption and in both cases, severe rectal irritation developed when the dose reached 30 gm. of potassium iodid daily. According to these results, it appears that approximately only 20 to 25 per cent. of iodid given by rectum is absorbed; since 96+ per cent. is absorbed after oral administration, a daily dose of 2 to 3 gm. (30 to 45 grains) by mouth is equivalent to 10 gm. (150 grains) by rectum. Therefore, it would seem that the rectal administration of iodids offers no particular advantage in the treatment of disease.

Mechanism of Absorption and Circulation of Iodids.—The iodids being freely soluble are doubtless absorbed directly into the lymph and venous blood through the epithelium of the glands of mucous membranes. The studies of Osborne have suggested, however, that after absorption of potassium iodid an exchange of ions occurs in that the potassium is replaced by sodium to a large extent. For example, after the ingestion of 1, 5 and 20 gms. of sodium iodid, Osborne found that a definite rise in the sodium content of the blood proportional to the size of the dose; but after the ingestion of similar amounts of potassium iodid, no such change in the potassium content of the blood occurred except a slight increase after the 20 gm. dose. On the contrary, a rise in the sodium of the blood was observed. After intravenous injections of 10 gm. of sodium iodid, the sodium content of the blood remained high for twelve hours, and returned almost to normal at the end of forty-eight hours. After the injection of the same amount of potassium iodid, however, no such increase in potassium occurred. Osborne then observed after the ingestion of sodium iodid, that only traces of iodin were found in the serum protein, while after intravenous injections from 6 to 9.9 per cent. of the iodin was found in these proteins. After the ingestion of potassium iodid, however, an average of 16.8 per cent. of iodin was found in the serum proteins. Analyses of the blood serum lipoids showed only traces of iodin in the alcohol- ether- and chloroform-soluble fractions after potassium and sodium iodid by mouth and sodium iodid intravenously.

The significance of these observations in relation to the toxicity of the iodids must yet be determined, but it would appear that, after the administration of sodium iodid, the whole molecule is absorbed without dissociation of the sodium or union with the proteins, whereas after the administration of potassium iodid, the potassium is absorbed only to a slight degree while the iodid ions unite with the serum proteins to form a sodium protein combination. Possibly changes of this sort may account in part for the higher toxicity of potassium iodid and such effects as higher hemolytic activity. After absorption of the iodids, the large portion circulates in ionic form, passes through the tissues in this form and is largely excreted in the urine in the form of salts. However, a small amount evidently undergoes decomposition into free iodin, since some may be found in the stomach and an increase sometimes occurs in the thyroid gland after the administration of potassium iodid. Furthermore, an organic compound of iodin is known to occur in the hair and in various internal organs particularly the liver, after iodid therapy. However, free iodin thus liberated does not circulate as such, but in combination with the plasma proteins, as above stated; insofar as the treatment of syphilis and other infections is concerned, only the iodid ion and not either free iodin or organic protein-iodin compounds are of particular interest.

Elimination of Iodids in Relation to Methods of Administration.—Iodids are mostly eliminated in the urine, with traces in the saliva, tears, perspiration, milk, sebum, nasal discharges and gastric juice; in the latter, they occur

as hydriodic acid, from which free iodin may be liberated. Only very small amounts are eliminated in the feces. Osborne found that, following a dose of 5 gm. of sodium iodid, 0.2 gm. of iodin was found in the feces, and after a dose of 20 gm. of potassium iodid, 0.250 gm. of iodin appeared in the feces. Thus, 96 per cent. was absorbed from the gastro-intestinal tract, and this in spite of the fact that 20 gm. doses produced a mild watery diarrhea in some of his cases. About 1 per cent. of iodid is eliminated in the bile in twenty-four hours, the maximum in the first three hours, but apparently re-absorption occurs to some extent in the lower intestine. Elimination also occurs in other fluids, as transudates and inflammatory exudates; of special interest, from the standpoint of the treatment of neurosyphilis, is the occurrence of iodid in the cerebrospinal fluid.

In the urine the iodids are eliminated largely as iodid ions; a part is also apparently eliminated as salts and in organic combinations, but the evidence of the latter is somewhat controversial and unconvincing. They appear in the urine in ten to twenty minutes after ingestion and reach a maximum in from two to four hours; no appreciable differences are noted in the rates of elimination when the drugs are given before or after ordinary meals. About four-fifths is excreted within a few hours and the balance, except for traces, by the end of ninety-six hours regardless of the dose. The amount of urine excreted does not materially influence the iodin output.

Salivary excretion begins usually in six to eleven minutes, being somewhat delayed when taken on a full stomach. A metallic taste is imparted to the saliva, best corrected by gargling with bicarbonate of soda, but there is rarely an odor of the breath, although patients and particularly women, sometimes object to iodid therapy because of a suspicion of halitosis.

Elimination in the milk, known to Fournier, and studied with particular care by Reipt and Scheffer,[5] is of particular interest in the treatment of syphilis of infants, since it is possible to administer the drug to them by this route through their mothers or wet nurses in much the same manner as mercury or arsphenamin and its substitutes.

According to Osborne, sodium iodid is excreted faster during the first hour than potassium iodid. The percentages for the one-hour interval following the administration of 1 gm. of sodium iodid and 1 gm. of potassium iodid, respectively, were 5.3 and 2.2; 7.3 and 7.2 after 5 gm., and 10.9 and 2.0 after 20 gm. Other pharmacologists, however, found that the cathions had no influence upon rapidity of elimination, and that sodium, potassium and ammonium iodids are excreted precisely alike, both in regard to rate and total quantity. The administration of related ions, like chlorids and bromids, however, apparently exert an influence, since some pharmacologists have found that chlorids and related ions hasten elimination while foods poor in chlorin increase retention. However, the excretion of iodin after the ingestion of the iodids is about the same for all compounds, although Osborne observed that the percentages were on the average a trifle higher with sodium iodid. Of particular interest in relation to methods of administration is the comparative rates of elimination after intravenous and oral administrations; I may quote Osborne in this connection, who states on the basis of a study of results following doses of 5, 10 and 20 gm. of sodium iodid: "(1) There is no striking difference in the rate of elimination of sodium iodid given intravenously, as compared with that given by mouth. Mendel has contended that sodium iodid administered intravenously is retained in the body for a longer period. My observations indicate that there is practically no difference between the two methods in the rate of elimination, and large doses are eliminated as rapidly as small ones.

"(2) There is a rapid adjustment of the body fluids to the hypertonic sodium iodid solution (10 per cent.). After 5 gm. of sodium iodid intravenously, the concentration of iodin in the urine rises immediately; while, after the 10 gm. dose, it is a trifle slower. With the relatively large dose of 20 gm., the urinary concentration remains comparatively low until the end of the first hour, after which it rapidly rises to a maximum during the fourth hour. Clinical experience coincides with these findings. Practically every adult patient tolerates 10 gm. of sodium iodid intravenously (100 c.c. of 10-per cent. solution) very well because the body is able to adjust itself to that amount. Above 10 gm., symptoms may develop from the effect of the hypertonic solution. No accumulation of the drug in the body after the large daily doses intravenously occurs. On 30 to 45 gm. daily, the same percentages were obtained as with small doses, and the drug did not remain in the body any longer."

Retention and Distribution of the Iodids in Relation to the Treatment of Syphilis and Other Diseases.—As previously stated, only a small part of the iodids administered are retained; fully 65 to 80 per cent. appears in the urine within twenty-four hours after their administration, and no iodid reaction is obtained from any of the secretions a week after treatment has ceased. The fate of the retained iodid is not, however, clearly established. In the first place the amounts retained varies with different individuals; thus as stated by Cushing, one patient receiving 0.5 gm. of potassium iodid may retain 0.1 gm., while another after the same dose may retain 0.2 gm. or more, the same proportions appearing on different occasions.

The largest amounts are apparently retained in the thyroid gland followed in order by the blood, secretory and lymph glands, kidneys, lungs, muscles, intestines, testes and bones. Only traces occur in the hair and none in the brain, spinal cord or bone marrow (except traces if the lipoid-soluble organic forms are given). Recently Steiglitz,[6] who worked out a histological method for studying the distribution of iodids, found them chiefly in the secreting cells of parenchymatous organs such as the liver and kidneys; also in the epithelium of the mucosa of the trachea and bronchi. McLean found after the administration of potassium iodid and iodalbin, that 68 per cent. of the iodin was present in the water soluble fraction of the liver, 32 per cent. in the lipoid, and none in the protein. After the administration of sajodin (iodized fat), however, 58 per cent. was found in the lipoid fraction and 42 per cent. in the water-soluble fraction.

Of special interest in the distribution of the iodids are their presence in the cerebrospinal fluid and inflammatory exudates, especially the exudates and gummas of syphilis. Catton,[7] in a study of the spinal fluid of four patients who had taken 15 grains of potassium iodid three times a day for periods varying from seventeen days to three months, found none in the spinal fluids. Mehrtens[8] found that spinal fluid drawn one hour after the last of four intravenous injections every half-hour of 50 grains of sodium iodid contained no iodin, but cases previously treated with an intraspinal injection of 10 c.c. of sterile horse serum showed traces of iodin, presumably due to an increased permeability of the choroid flexus and vascular changes of the meninges as the result of irritation by the horse serum. A very comprehensive study by Osborne,[9] however, has shown that iodin (determined by Kendall's method) is found in normal spinal fluid (0.000019 to 0.000024 gm. per 100 c.c.) and undergoes an increase after the administration of iodids by various methods. After the ingestion of potassium iodid in dose of 50 to 100 grains three times a day, spinal fluids drawn two to seventy-two hours after the last dose showed from traces to 0.00037 gm. per 100 c.c. After the rectal injection of 125 to 200 grains of potassium iodid by rectum three times a day, spinal fluid drawn one to twelve hours after the last dose showed 0.000381 to 0.0018 gm. per 100 c.c. After the intravenous injection of 10 to 20 gm. of sodium iodid in 10-per cent. solution to normal individuals, spinal fluid drawn fifteen minutes to forty-eight hours later showed amounts of iodin varying from 0.0027 gm. (two-hour interval) to 0.0003 gm. (twenty-four-hour interval) per 100 c.c. of fluid. In a series of three cases of

neurosyphilis given daily intravenous injections of 10 to 40 gm. of sodium iodid for two weeks with spinal puncture half to twenty-four hours after the last dose, as much as 0.0048 to 0.0423 gm. of iodin per 100 c.c. of fluid were found. In one case of neurosyphilis receiving one intravenous injection of 20 gms. of sodium iodid, as much as 0.00437 gm. of iodin per 100 c.c. of spinal fluid was found two hours later. Osborne concluded that iodin in increased amounts is present in cerebrospinal fluid following the administration of iodids by mouth, by rectum and intravenously and especially after the last route of administration. His work also suggests that neurosyphilitic tissues take up more iodin than normal tissue and that the presence of syphilitic meningitis increases the permeability of the choroid plexus and meninges to iodin compounds in the blood.

Loeb[10] had previously shown that syphilitic lymph glands retain more iodid than normal tissues, and this increased retention of iodids by syphilitic tissues is a matter of considerable significance from the standpoint of chemotherapy, since it probably opens up a means for bringing spirocheticidal agents in contact with the parasites. Of equal importance are the studies showing that tuberculous tissues and particularly the necrotic portions, contain increased amounts of iodids since advantage may be possibly taken of this to effect some combination between iodid and tuberculocidal drugs and thereby favor the more direct access of the latter to the tubercle bacilli. Carcinomata and various granulomata as in actinomycosis, sporotrichosis and other necrotic tissues likewise show an increased retention of iodids. Just how or why this affinity exists between iodids and the inflammatory exudates of syphilis, tuberculosis, actinomycosis, etc., cannot be stated. Evidently there is nothing specific about the process, since increased amounts of iodids are to be found in the necrotic tissues produced by various bacteria and indeed, by sterile chemical irritation; evidently it is a matter largely of simple diffusion in which dying and dead cells no longer offer resistance to the diffusion of the salts. Apparently the iodin exists in syphilitic and tuberculous tissues as organic compounds of lipins rather than of proteins, as shown by Fujisawa;[11] in pus, however, and exudates generally, the iodin has been found as inorganic iodids.

Influence of Iodids upon Pathological Tissues and Secretions and the Mechanism of Their Therapeutic Activity.—This naturally raises the question what effect the increased retention of iodids may have on pathological tissues and exudates and the course of subsequent events. The chief effect is one of softening and liquefaction of the diseased tissues and secretions. The results of iodid therapy in tertiary syphilis are sometimes little short of marvelous, insofar as the rapid disappearance of gummas is concerned. This does not mean that the spirochetes are necessarily killed by the iodids; indeed, available evidence indicates that the iodids are very feeble spirocheticidal agents, as reviewed in Chapter XXVI, but I am not so sure but that the products of protein digestion, in the nature of the products of liquefaction necrosis, are sufficiently spirocheticidal, as they are known to be bactericidal, to possibly sterilize gummas. At least, they facilitate the access of such spirocheticidal agents as the oxids of arsphenamin and its substitutes, mercury and bismuth, if they happen to be present in the body. For this reason I believe that large doses of the iodids in syphilis should always be preceded and accompanied by the administration of arsphenamin, neoarsphenamin or one of their substitutes, mercury or bismuth, to secure the maximum therapeutic results and to prevent the possible dissemination of living spirochetes; doubtless, if we had tuberculocidal and leprocidal agents of equal potency, the administration of the iodids would be much less dangerous in tuberculosis and leprosy.

Likewise, in tuberculosis, leprosy, actinomycosis, sporotrichosis, blastomycosis, etc., there is usually observed a breaking down of the granulomatous tissues with liquefaction of the exudates and secretions; indeed, for this reason, the iodids are usually contraindicated in tuberculosis and leprosy, because of the danger of disseminating the bacilli, since no means are available for bringing about their simultaneous destruction such as we possess in syphilis. Bronchial secretions are likewise liquefied, and for this reason the iodids are frequently useful in the treatment of bronchitis and bronchiectasis, with or without asthma, and especially when the secretions are very heavy, viscous and difficult to "get under" for expectoration.

How do the iodids produce these results? Why do they not act in a similar manner upon normal tissues? The second question is probably more difficult to answer than the first, but it is apparently due to the fact that tissues devitalized by spirochetic, bacterial or sterile chemical agents (as intracutaneous injections of agar, luetin, etc.) absorb more iodid than normal tissues and that the difference is largely one of quantitative variations. For my own part, I believe that the answer to the first question is to be ascribed to the effects of an increase of proteolytic activity in the pathological tissues, secretions and exudates, as explained by Jobling, Petersen and Eggstein,[12] along possibly, with an actual increase of proteolytic ferments, as suggested by Dick. Certainly the older theories of liberation of free iodin (Binz), increased leukocytosis, phagocytosis and capillary permeability (Heinz), salt action and effect upon the thyroid gland, etc., are entirely obsolete or too general and indefinite to explain these specific effects of the iodids. According to Jobling and his colleagues, the proteolytic ferments of cells are held in physiological check or equilibrium by the unsaturated carbon atoms of the lipoids of the plasma constituting the antiferment or antitrypsin of the body fluids. The administration of the iodids and other halogens as well is followed by the saturation of these carbon groups, which thereby removes or reduces antitryptic activity, followed by increased proteolysis by the proteolytic ferments normally present in all cells as well as in the blood plasma and other tissue juices. This increased proteolytic activity results in a digestion of the dead protein material and of such cells as are so far devitalized as to be vulnerable to this increased digestive activity. The result is that inflammatory fibrous tissue (gummas, the shells of tubercles, etc.) undergo softening and liquefaction, as well as the exudates and secretions, followed by absorption, if their escape is otherwise impossible.

While the most marked and spectacular effects of the iodids are observed in tertiary syphilis, their administration is also indicated in the treatment of early syphilis for their effects upon exudates not yet advanced to the stage of fibrosis. Their use, however, is not limited to syphilis, since they are useful in other infections as well. It is to be noted, however, that the iodids have no influence upon completely healthy tissues; therefore, their administration does not influence ordinary scars and possibly not even the scar tissue of infectious processes, if the cells are entirely free of the effects of bacterial toxins or other agents and to all intents and purposes normal tissue even though the result of originally pathological processes.

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CHAPTER LVII

TOXICOLOGY OF THE IODIDS IN RELATION TO THE TREATMENT OF SYPHILIS AND OTHER DISEASES

Fortunately the iodids are much less toxic than the organic arsenicals, mercurial and bismuth compounds employed in the treatment of syphilis, and consequently they are less dangerous and their administration is not followed as frequently by ill effects. Some individuals, however, possess a natural hypersensitiveness to the iodids so that even small doses may produce toxic reactions and, since most cases of syphilis or other diseases calling for iodid therapy require a prolonged administration of the drugs, ill effects are likely to develop in any case; while these are seldom dangerous, they may be very disagreeable and the whole subject is worthy of close attention in relation to the iodid therapy of disease.

Toxicity of the Iodids for the Lower Animals.—Enormous doses of sodium or potassium iodid may be administered to rabbits and rats by stomach tube without any discernible ill effects other than progressive loss of weight and emaciation upon prolonged administration. This is apparently largely due to disturbances of the gastro-intestinal tract with loss of appetite, as well as to an influence upon the thyroid gland and metabolism.

By intravenous injection, the maximum tolerated dose of potassium iodid for white rats has been in my experience about 0.310 gm. per kilo of weight administered as a 4-per cent. solution in water; this corresponds to over 18 grams per 60 kilograms or approximately 132 pounds. Potassium iodid is seldom administered to human beings by intravenous injection, preference being given the less toxic sodium iodid, but when injected, the dose should not exceed 2 or 3 grams in order to leave a good margin of safety.

Sodium iodid is about eight times less toxic since the maximum single tolerated dose administered as a 60-per cent. solution, is about 2.4 gm. per kilo which corresponds to the enormous amount of more than 144 grams per 60 kilograms or 132 pounds. Since the maximum dose injected intravenously in the treatment of human beings is seldom more than 20 grams, it is apparent that this dose is at least seven times less than the toxic dose for rats. Sodium iodid, therefore, is always to be preferred for intravenous injection.

By repeated administrations to rabbits, however, the toxicity of sodium and potassium iodids are about the same. For example, rabbits have stood daily amounts of potassium iodid by stomach tube as large as 0.24 gm. per kilo for 36 doses in succession; curiously, sodium iodid was less well borne by this route as the majority of animals succumbed. By intravenous injection rabbits have stood bi-weekly injections of potassium iodid in dose 0.10 gm. per kilo for 12 injections; sodium iodid was tolerated in about the same degree.

Just why there is this big difference in the toxicity of single doses of potassium and sodium iodids by intravenous injection is not definitely known. Possibly it is due to the more rapid elimination of sodium iodid or to the fact that the potassium ion is dissociated and more toxic than the sodium ion. Of interest in this connection is the fact that I have found sodium chlorid tolerated by white rats injected intravenously in doses as high as 1.5 gm. per kilo in 10-per cent. solutions, whereas potassium chlorid was 30 times more toxic being tolerated in doses of only 0.050 gm. per kilo. Sodium chlorid in dose of 2.0 gm. per kilo was immediately lethal; potassium chlorid was immediately lethal in dose of 0.1 gm. ker kilo. These results suggest that the chief reason for the great variation in toxicity of sodium and potassium iodids is the greater toxicity of the potassium ions. As will be discussed presently, potassium iodid is likewise more hemolytic than sodium iodid which may be an additional reason for its greater toxicity. Clinical experience has shown that potassium iodid and sodium iodid are tolerated about equally well by ingestion; possibly sodium iodid is less irritant and better tolerated. It is to be noted, however, that the toxicity of both compounds for rabbits by repeated intravenous injections and by ingestion does not vary nearly as much as by single intravenous injections.

Pathological Tissue Changes Produced in the Lower Animals by the Iodids.—As stated in Chapters XLVII, LII, and LV, the administration of arsphenamin and its substitutes, mercury compounds and bismuth compounds may result in important tissue changes in the internal organs of great interest in relation to chemotherapy and of practical importance in relation to the treatment of syphilis. There is much less reason for expecting, from clinical experience, that the iodids produce pathological tissue changes, but since they are frequently administered in large doses over prolonged periods of time, Lucke and I thought it well worth while to study the histological effects produced by potassium iodid and sodium iodid administered to rabbits by ingestion and intravenous injection.[1] Very little work has been done in this field, although Boehm[2] and Sgalitzer[3] have touched upon it briefly in pharmacological studies with the iodids. The results of our studies have shown that the administration to rabbits by stomach tube of potassium and sodium iodid in repeated large doses equivalent to approximately as much as 15 gm. per 60 kilograms of body weight, and repeated intravenous injections in amounts approximately equivalent to as much as 1 gm. per 60 kilograms, as well as single intravenous injections in amounts equivalent to approximately 30 gm. per 60 kilograms, did not cause tissue changes in the brain, heart, and suprarenals. The kidney, liver, lung and spleen showed slight tissue reactions which may be interpreted as the result of a mild irritation. Dosage, compound and route of administration did not produce any essential differences in the histologic changes.

An Etiological Classification of the Toxicological Effects of the Iodids.—It is apparent, therefore, that both potassium and sodium iodids do not produce any chemopathological tissue changes of note in the internal organs when administered in ordinary doses even over long periods of time. The iodids, however, are not lacking in local and general toxic effects, but we have but little information on the nature and mechanism of their production although their clinical manifestations are well known. Among the known toxicological factors may be mentioned the following:

1. Direct irritation of mucous membranes as of the gastro-intestinal canal by ingestion due to the iodids as such, to free iodin or impurities.
2. Irritation by dissociated ions and particularly by potassium after intravenous injections of potassium iodid.
3. Irritation of excretory organs as the mucosa of the respiratory tract, conjunctivæ, salivary glands, gums and skin.
4. Interference with excretion and particularly by nephritis.
5. Effects upon metabolism and the thyroid gland.
6. Intravascular hemolysis when iodids are injected intravenously in large doses.
7. Increased proteolytic activity with liquefaction necroses in areas of infection.
8. Production of Herxheimer reactions.
9. Iodid hypersensitiveness.

These factors may be now briefly discussed with more detail.

Direct Iodic Irritation.—That the iodids are capable of exciting irritation of mucous membranes with congestion, edema and serous exudation is seen clinically by gastric disturbances of pain, nausea and vague distress after ingestion. Concentrated doses in powders, pills, cachets, tablets, etc., produce much more irritation than diluted solutions and the last mentioned are always preferred for administration. Intestinal irritation is much less common, but does occur even to the extent of producing colic and diarrhea. The effects of local irritation are also to be observed when the iodids are administered by rectal injection.

It is possible that some of these effects are due to the liberation of free iodin in the stomach; also to the presence and effects of iodates.

When potassium iodid is injected intravenously the depression of the central nervous system and heart are generally ascribed to the potassium ions since the effects do not seem to be more poisonous than other potassium salts; at least these disturbances are produced only by very large doses in the lower animals and are not ordinarily observed in human beings.

Very probably the iodids are also capable of producing a similar irritation of the organs and cells concerned in their elimination and in this manner produce salivation by irritation of the salivary glands and especially the parotids; stomatitis, by irritation of the buccal mucosa; coryza, by irritation of the nasal mucosa; bronchitis and faucial edema, by irritation of the pharyngeal and laryngeal mucosa; conjunctivitis, by irritation of the conjunctivæ and a whole host of skin disorders, by irritation of the sweat and sebaceous glands.

Anything interfering with elimination by the kidneys and liver, and especially nephritis with retention, naturally throws an added weight upon the skin and these mucous membranes, which tends to increase the local irritant effects.

Iodic Disturbances of Metabolism and of the Thyroid Gland.—Naturally, if these effects are prolonged, one may expect to observe a loss in weight, anorexia, mental depression, irritability, loss of sexual power, etc., along possibly with nervous excitation and tachycardia due to disturbances of the thyroid gland. Indeed, one must exercise due care in the selection of patients for intravenous injections of sodium iodid, insofar as the thyroid gland is concerned, in order to avoid accidents from this source; in this connection I may mention that Schaffer[4] has recently observed the precipitation of hyperthyroidism in a patient with unsuspected adenomas of this organ following intravenous injections of sodium iodid and he advises extreme caution in the presence of even the smallest palpable nodule in the gland.

Otherwise, however, the metabolism of patients is not liable to suffer. Chistoni[5] found that there may be some increase of urea, total nitrogen, uric acid, purin bases and chlorid excretion and recently Crabfield[6] has stated that multiple small doses of both potassium and sodium iodid may double and treble the nonprotein nitrogen of the blood, the increase beginning about the second or third day, continuing for four or five days, and thereafter gradually returning to the previous level; the chlorids remained unchanged. Single doses were without these effects, which were apparently associated with an adjustment of the body fluids to the iodid in the blood and probably with some renal retention of nitrogen.

Iodic Hemolysis.—When potassium or sodium iodids are given by intravenous injection, it is possible that some minor reactions may be produced by the effects of hypertonic solutions, to proteins in the water and to intravascular hemolysis. I have found that 2 c.c. of 1:24 solutions of potassium iodid in saline causes the partial hemolysis of 1 drop of unwashed human blood while sodium iodid is less hemolytic (1:8). Washed corpuscles are much more susceptible and it may be that the serum effectually prevents intravascular hemolysis altogether; at least intravenous injections of human adults with 100 to 200 c.c. of 10-per cent. solutions of sodium iodid have not produced reactions suggesting sufficient hemolysis to produce lesions and symptoms. Agglutination of corpuscles does not occur.

Iodic Proteolysis.—Since the iodids are especially retained by devitalized pathological tissues and their exudates and secretions, it is not surprising that some of the toxicological effects of the iodids are due to increased pro-

teolysis of these substances as the result of removal of antiferment by the saturation of the unsaturated fatty acids by iodin. I believe that this accounts for the occasional appearance of urethral discharges in cases of chronic gonorrhea, the increased coryza and bronchitis of chronic rhinitis and bronchitis, the lighting up of latent foci of infection of joints with articular and periarticular swellings, the lighting up of latent infections of nerves with the production of neuritis, etc., sometimes following the administration of iodids in the treatment of syphilis and other diseases.

Iodic Jarisch-Herxheimer Reactions.—It is possible that the iodids may intensify syphilitic lesions, not only by a direct stimulation of spirochetes, but more indirectly by their effects upon syphilized tissues. These results, however, are not at all probable when the usual small doses of the iodids are given by ingestion. The iodids do not ordinarily increase the degree of complement fixation in syphilis or the cells in the spinal fluid in neurosyphilis, which are probably more sensitive indices of stimulated spirochetic activity than clinical lesions and symptoms. On the other hand, the cutaneous lesions of syphilis may undergo exacerbation during iodid therapy and it is possible for Herxheimer reactions to occur, but not with any thing near the frequency with which they may be observed after the administration of arsphenamin and its substitutes and mercury.

Iodic Hypersensitiveness.—And finally, one of the most important and frequent causes for untoward effects from the iodids is natural hypersensitiveness, analogous to the natural hypersensitiveness displayed by some individuals to arsenic, mercury, quinin, etc. The literature contains numerous descriptions of cases in whom iodism was brought on by very small doses of the iodids administered by ingestion. Some of the earliest experiments on the mechanism of drug allergies were inspired by clinical examples of hypersensitiveness to iodoform and other iodin compounds with the general result that it has been shown that guinea-pigs may be sensitized and shocked by injections of guinea-pig serum previously iodized in the test-tube. Friedberger and Ito[7] even claim that guinea-pigs sensitized with iodized guinea-pig serum may be shocked by injections of sodium iodid, iodoform and Lugol's solution; likewise, that guinea-pigs may be sensitized by injections of Lugol's solution to injections of iodized serum, but not to Lugol's solution itself. Indeed, more work has been done on iodid and iodin hypersensitiveness than with any other form of drug allergy, but all attempts have failed to transfer the hypersensitive state from human beings to guinea-pigs by injections of human serum.

Apparently, hypersensitiveness to the iodids may be acquired; at least, one sometimes observes marked intolerance after iodids have been taken for some time and even after periods of rest, when one may be reasonably sure that the effects are not due to accumulative toxicity. Curiously enough, large doses are sometimes better borne under these conditions than small doses, a fact to which I will again refer in more detail in Chapter LXV devoted to a consideration of methods of administration of the iodids.

Clinical Classification of the Toxic Reactions Produced by Iodids.—In the great majority of instances the toxic manifestations of iodism are mild and comprise a slight degree of salivation with a metallic or salty taste of the saliva, coryza and a few scattered acneiform lesions of the skin of the face and neck. These effects are apparently due to the excretion of the iodids through the salivary glands and particularly the parotids, and the mucosa of the upper respiratory tract. It is an easy matter, however, to overlook mild symptoms of this kind or the patient may fail to report them or coöperate properly with the physician, in which case severer and even dangerous symp-

toms may develop if the administration of iodids is continued. Likewise, severe and even alarming lesions like faucial edema may develop in those hypersensitive to the iodids even after the ingestion of small amounts.

There is, therefore, quite a long list of the possible toxic reactions produced by the iodids, but since permanent tissue injuries are uncommon, most of these quickly disappear when the administration of the drug is topped. Furthermore, the majority are uncommon and especially the severe lesions. They may be classified as follows:

1. *Disturbances of the Digestive and Accessory Organs*
   - (1) Metallic or salty taste
   - (2) Slight salivation
   - (3) Parotid irritation and "iodic mumps"
   - (4) Stomatitis
   - (5) Gastro-intestinal irritation
2. *Disturbances of the Respiratory and Accessory Organs*
   - (1) Rhinorrhea
   - (2) Coryza and "iodic grippe"
   - (3) Conjunctival catarrh
   - (4) Faucial edema
3. *Disturbances of the Skin*
   - (1) Iodic acne
   - (2) Iodic erythema, urticaria, bullæ, etc.
   - (3) Iodic purpura
4. *Disturbances of the Thyroid Gland (Hyperthyroidism)*

This list appears quite formidable and may readily enough give the impression that the iodids are dangerous drugs capable of producing a host of ill effects. But it includes the more uncommon toxic manifestations and, as previously stated, iodism in practice seldom proceeds beyond the stage of mild coryza, a mild acne and a few vague pains and "tightness of the jaws" in the neighborhood of the parotid glands. But if one were to include in a classification even a part of the large number of accidents and ill effects ascribed to the iodids in the literature, like hemiplegia, impotence, nephritis, etc., the list would be even larger; many of these, however, are now known to be due to syphilis or mercury and erroneously ascribed to the iodids which may have been given at the time; or they have been due to the physiological effects of the iodids upon pathological tissues without being toxic effects, although readily enough interpreted as being toxic manifestations.

Symptoms and Treatment of Toxic Iodid Reactions.—*Iodic taste* is a disagreeable salty or metallic taste, most common in the morning and especially complained of by women. It is due to the excretion of iodid in the saliva. Halitosis or odorous breath is not usually present although the patient frequently suspects that it is on account of the taste. It rapidly disappears when the administration is stopped or the dose reduced.

Iodic salivation is usually slight and mild, without halitosis or inflammatory changes of the gums and buccal mucosa. It is due to irritation of the salivary glands, and especially the parotids, by the excretion of iodids.

Parotid irritation is usually in the form of diffuse vague pains at the angles of the jaw. Women are especially likely to complain of vague neuralgic pains or "tightness" about the jaws which are difficult to describe or locate. Sometimes the pains are located in the gums, although the teeth and gums appear healthy. I believe that these are due to irritation of the parotid, sublingual and submaxillary glands by the excretion of iodid. They may make it impossible for the patient to take more than a very small daily dose; they usually disappear quite promptly when the administration is stopped. Rarely, fluctuating swellings of the glands occur which I ascribe to hypersecretion with blockage of the ducts; when the parotids are so involved the condition is frequently designated as "iodic mumps."

Iodic stomatitis and *gingivitis* are quite rare, because the iodids are not deposited in the buccal and gingival mucosa as are mercury and bismuth. Inflammatory changes and even ulceration may occur, however, in severe neglected cases of iodism or in cases of hypersensitiveness to the iodids. The lesions quickly disappear upon withdrawal of the drug and the use of simple mouth washes.

Gastro-intestinal irritation is expressed in the form of vague abdominal "uneasiness" or pain, along with anorexia, coated tongue and headache. Constipation or diarrhea may be present and the salty, metallic taste of iodid is especially disagreeable. These symptoms are not produced by intravenous injections and are apparently due to direct irritation of the gastro-intestinal mucosa by the drug administered by ingestion. In treatment constipation and dietary errors should be corrected, if present. The dose may require reduction although, curiously enough, the symptoms may disappear when the dose is increased. It is sometimes advantageous to give the drug about one-half hour before meals instead of after meals as is the usual custom. A special effort should be made to disguise the taste of the drug; I have found it advantageous to give it in a small glassful of cold ginger-ale or sarsaparilla.

Iodic rhinorrhea is the most common symptom of all; indeed, few patients escape it when large doses are given. In mild form the discharge is thin and serous and seldom muco-purulent. The nose is obstructed, due to congestion and edema of the mucosa and this may produce sneezing and radiating pains in the frontal sinus, headache, etc. as in hay-fever. Due care must be exercised in administering iodids to individuals with sinus infections. In its milder forms it is nothing more than an inconvenience and may pass away spontaneously; it is generally necessary to stop the drug for a few days and then resume with a smaller dose.

Iodic coryza, however, may develop and especially if the patient is hypersensitive to the iodids. This condition may be serious, inasmuch as the nasal discharge and swelling of the mucosa may be excessive, with a great deal of sneezing, edema and congestion of the eye lids, violent headache, etc. I have known sinus infections to follow and in some cases, latent infections of the antra, frontal and ethmoid sinuses have been aggravated. Treatment consists in promptly stopping the drug. In some cases it is necessary to reduce the turgescence by spraying the nose with a weak solution of adrenalin chloride like equal parts of 1:1000 solution and rose water with 10 grains of antipyrin to the ounce. Small doses of belladonna or atropin may be helpful in the form of the usual coryza tablets.

Conjunctival catarrh and iodic edema of the face usually develop in cases of severe iodic coryza. The eye-lids and conjunctiva are red, swollen and may itch or smart as in hay-fever, along with a thin watery secretion. A peculiar but characteristic edema of the tissues below the eyes may develop along with redness of the nose; the edema and redness of the eye-lids, associated with the conjunctival changes and erythema of the nose, fever, headache, fulness of the head and shooting pains, sometimes resembles an outbreak of urticaria or even erysipelas. It is difficult to explain these lesions; I have considered them due primarily to blockage of the lacrymal apparatus and conjunctival drainage by the involvement of the nasal mucosa. Possibly they are due in small part to irritation of the lacrymal glands by iodid since the drug may be found in the tears. Fortunately the lesions and symptoms quickly disappear when the drug is stopped.

In exceptional cases a train of symptoms like coryza, epiphora, sore throat, dyspnea, mild fever, malaise and severe headache resembles an attack of

influenza or "grippe" and are sometimes designated as "iodic grippe." The condition may be readily enough mistaken for an attack of tonsillitis or influenza, but the body pains are usually absent, the condition develops suddenly and subsides rapidly when the drug is stopped. In rare instances of extreme hypersensitiveness to the iodids the edema may involve the uvula, glottis and fauces in general, producing *acute laryngeal and pulmonary edema* characterized by dyspnea, stridor, cough, etc. Most recorded cases have developed after the first and second doses and with amounts as little as 3 to 15 grains. Tracheotomy has been required in some instances. I have never seen a case of this kind, but surmise that intramuscular injections of 0.5 to 1 c.c. of 1:1000 adrenalin may give relief.

Iodic acne is one of the most common manifestations of iodism and in mild form consists of a few scattered red pimples with pustular tops usually in the skin of the face and neck and rarely on the trunk and extremities. They tend to recur, but may disappear spontaneously even when the dose is raised. In some cases, however, these acneiform pustules become so large and numerous that the dose must be sharply reduced or the administration stopped, at least temporarily. In exceptional cases the eruption is purpuric (*iodic purpura*) with a predilection for the anterior surface of the legs and occurring symmetrically as discreet, miliary petechiæ of deep red color, painless, without itching and not disappearing under pressure. Occasionally the eruption is *bullous*, *eczematous*, nodular and tuberose, furuncular, fungoid, ulcerative or polymorphous. Due care must be exercised in differential diagnosis from syphilids, in which the sudden onset, forms, bright red areolas, softer bases, etc., of the iodic eruptions, along with their rapid disappearance when iodid ingestion is stopped, are useful differential points.

The cause of these eruptions has been a subject of much speculation and some investigation. They have been usually ascribed to irritation of the sweat and sebaceous glands by free iodin supposed to be liberated from the iodid by chemical action in these structures. Some investigators state that iodid is to be found in the pus of the lesions but Wile, Wright and Smith[8] have recently stated that this is an error probably due to contamination with iodid in the sweat.

As pointed out by Engman and Mook,[9] iodid acne is especially likely to develop in seborrheic and acne areas and areas of previous inflammation or injury; in their opinion the glands and follicles of the skin take no specific part in the production of the lesions. They suggest that iodids may cause some disturbance of equilibrium in the blood with the production of a toxin, which in turn causes irritation and various local inflammatory lesions. Wile and his associates do not regard the lesions as due primarily to the excretion of iodid or the activities of bacteria, but to some undiscovered complex biochemical reaction.

In my opinion, the pustular lesions at least are due to direct iodid effects in the skin as a result of the presence of the drug in the glandular secretions acting upon tissues of lowered vitality as a result of predisposing injuries so small as easily to escape detection, as black heads and minor bacterial infections of the sweat and sebaceous glands, but sufficient to favor increased proteolytic activity in the devitalized cells as a result of reduction in antiferments by the iodids; this results in increased proteolysis, with consequent irritation due to the products of digestion plus those due to secondary infection of the lesions with the staphylococci of the skin. I believe this mechanism explains the production of the acneiform, furuncular, fungoid and ulcerative lesions, as it does the production of local pustular lesions in syphilitics and non-syphilitics alike when given intracutaneous injections of luetin or other

substances while under the influence of iodids. The erythematous, bullous and hemorrhagic lesions are probably due primarily to toxic effects upon the nerves and capillaries comparable to the production of arsenical dermatoses. As in the bromid eruptions, these various lesions may develop after the administration of iodids has been stopped, but they usually occur during their administration.

In the treatment of these cutaneous disorders it is generally necessary to stop the ingestion of drug along with eliminative measures and the internal administration of Fowler's solution. They are rarely dangerous, not nearly as dangerous as the arsenical dermatoses and especially dermatitis exfoliativa. In severe cases it may be worth while to try intravenous injections of 1 gram of sodium thiosulphate in 10 c.c. of sterile water or saline, daily, for three or four doses.

Disturbances of the thyroid gland are in the nature of *hyperthyroidism* with tachycardia, tremors, and the usual signs and symptoms of this state in general. They are apparently of rare occurrence and likely to develop only in those cases with various forms of thyroid enlargement and hyperactivity. In reading over some of the case reports of nervous disturbances ascribed to iodids, one is impressed with the possibility of these being really due to hyperthyroidism when occurring in mild degree although usually ascribed to the effects of iodids upon the nervous system. Other case reports on the effects of iodids upon the nervous system read as if they were the manifestations of syphilis and neural Herxheimer reactions.

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CHAPTER LVIII

JARISCH-HERXHEIMER REACTIONS AND SYPHILITIC RECURRENCES

In the preceding chapters mention was frequently made of Jarisch-Herxheimer reactions and syphilitic recurrences in connection with the toxic reactions sometimes produced by mercury, arsphenamin and its substitutes and bismuth. In view of the practical importance of these subjects in relation to the diagnosis of toxic reactions and their treatment, as well as the treatment of syphilis itself, it may be well to discuss the subjects more fully.

Jarisch-Herxheimer Reaction.—In 1895, Jarisch[1] published a long series of cases of syphilis presenting roseola in whom the eruption became more marked and distinct during the administration of 2 to 5 daily inunctions of mercury. Apparently, however, these observations attracted little or no attention and not even Fournier appears to have noted or commented upon thin occurrence. A few years later Jadassohn[2] published similar observations

and in 1902, Herxheimer and Kraus[3] published such a complete account of the phenomenon as to attract considerable attention. They noted that in cases of secondary syphilis the injection of the first large dose of an insoluble salt of mercury was sometimes followed in fifteen to twenty-four hours by a change in the appearance of the skin rashes in which the lesions became larger and more prominent. Roseolar spots, formerly not noticed, became apparent; erythematous papules resembled urticarial lesions and the rashes sometimes became confluent and changed in color from a dusky to bright red. The authors stated that the reaction occurred only after the first injection of a large dose of mercury and not after the second or subsequent injections and showed that it was not due to mercurial poisoning.

Soon after the discovery of salvarsan, Finger[4] described the reaction following injections in the secondary stage of syphilis and the reaction has since became widely known as the Jarisch-Herxheimer reaction.

It is now well known that the reaction may be also produced by the various salts of bismuth being employed at present in the treatment of syphilis, but injections of arsphenamin, neoarsphenamin and their substitutes produce the reactions more prominently and frequently than either mercury or bismuth, while they have not usually been produced by the iodids at all. The administration of mercury by mouth usually does not produce the reaction, as likewise small subcutaneous injections or mild inunctions; it is highly probable that for these reasons Fournier and other syphilologists apparently did not observe the phenomenon or passed over the reactions as due to the natural cause of syphilis.

The *original* Jarisch-Herxheimer reaction, therefore, referred only to an exacerbation of the skin and mucous membrane lesions of the secondary stage of syphilis, and when the reaction occurred after an injection of arsphenamin it was noted that the skin or mucous membrane at the site of the lesion became turgescent and might weep, particularly if it were an infiltrated lesion. Usually within two to four hours after injection a roseolar eruption appeared or, if present, it became more prominent and papular and was regarded as a toxic dermatitis of the erythema multiforme type. As a rule, however, the macular rash disappeared in six hours while pre-existing lesions remained larger, redder and occasionally edematous. The pigmented syphilids might become more prominent and while the mucous membrane lesions reacted less frequently, yet they might undergo similar changes.

The *modern* Jarisch-Herxheimer reaction, however, includes not only these skin and mucous membrane exacerbations of the secondary stage of syphilis, but also the exacerbation of tertiary lesions of the skin and mucous membranes and of the internal organs as well. Reactions of this kind have become particularly prominent since the widespread adoption of the organic arsenical compounds in the treatment of syphilis and their occurrence in important organs as the cranial nerves and other parts of the central nervous system, may be not only dangerous but likely to be interpreted as an expression of toxic effects of the drugs.

Clinical Manifestations of Jarisch-Herxheimer Reactions. (*a*) *In the Primary and Secondary Stages of Syphilis.*—The typical and original reaction refers to the exacerbation of the skin and mucous membrane lesions of the secondary stage of syphilis along with an exacerbation of the glandular lesions in some cases (enlargement with pain and tenderness); these reactions have been described above, are not dangerous and of relatively slight importance.

It is highly probable, however, that a similar reaction takes place wherever the spirochetes happen to be located elsewhere in the body and in the

meninges, may result in the production of headache, increased amounts of spinal fluid with an increase of cells and an increase of protein. Even in the primary stage of syphilis one may observe an increase in the activity of spirochetes in the secretions during the first one to six hours after an injection of arsphenamin, although an enlargement of the lesions with exacerbation of the inflammatory phenomena may or may not occur.

(*b*) *In the Tertiary Stage of Syphilis.*—Not infrequently, however, similar and clinically more important reactions may occur in the tertiary stage of syphilis. Sometimes when the treatment of chronic latent syphilis is begun by the intravenous injection of 0.4 to 0.6 gm. arsphenamin or 0.6 to 0.9 gm. neoarsphenamin, the patient complains of tenderness of the inguinal lymph-glands, of vague pains in the region of the heart or elsewhere and these have usually impressed me as being the result of exacerbations of low grade chronic inflammatory lesions in these tissues.

It is particularly important to bear in mind that in active tertiary syphilis the administration of these drugs in large doses may result in an exacerbation of the lesions. Gummata, when not open, may become swollen and turgid; when ulcerated, they may enlarge and discharge an abundant serous exudate. Such lesions occurring along the skeletal system, in the nose, etc., do no particular harm but lesions of the central nervous system may be dangerous. In tabes the reaction may consist of intense pains due to involvement of the posterior spinal roots, which appear from one-half to one hour after injection and may last for a day or two. Gastric crises may be precipitated and the pains prove so severe as to require morphin although it has been noted that a second smaller dose of arsphenamin may afford relief. In cerebral sphilis the reaction may be manifested by intense headache and an exaggeration of focal symptoms and paralyses. When a cranial nerve which passes through a bony canal is involved, the resulting swelling may lead to paralysis, as sometimes happens with the auditory and facial nerves.

Reactions of this kind may be confused with neuro-recurrences or neuro-recidives and indeed, the pathological changes appear to be identical in both. In Herxheimer reactions, however, the lesions are produced a few hours after treatment is given due to an exacerbation of inflammatory phenomena; in syphilitic recurrences the lesions follow some days or weeks after cessation of treatment due to a resumption of activity on the part of spirochetes which have escaped destruction.

In chronic syphilitic myocarditis the reaction is manifested by pain and precordial distress; in the liver, by the development of constipation, "dyspepsia," anorexia and jaundice or, at least, a shallow sub-icteric coloring of the skin and mucous membranes. General symptoms at this time may be vague aches and pains in the extremities, malaise, loss of appetite, etc., which are so likely to be ascribed to the direct toxic effects of the drug administered but which in fact may be due to Herxheimer reactions of this kind and which pass away as treatment is continued.

(*c*) *Provocative Wassermann and Spinal Fluid Changes as Results of Jarisch-Herxheimer Reactions.*—Since the Jarisch-Herxheimer reaction appears to be in part at least the result of increased spirochetic activity it is to be expected that the Wassermann reaction with blood and spinal fluid may become stronger if already positive or, become positive if previously negative, and now well known as the "provocative" Wassermann reaction, previously discussed in Chapter XLI. It is very commonly observed that weakly positive Wassermann reactions in chronic latent syphilis become much stronger as treatment is given; I do not think that this is due to an effect of the drug upon the tissues with the production of such changes in

the blood as result in falsely positive reactions; rather I believe the reactions are due to an increased production of the Wassermann antibody or "reagin" as a result of increased spirochetic activity. It is much more common to observe weakly positive reactions becoming stronger after the first few doses of arsphenamin or neoarsphenamin, than to see negative reactions reversed to positive; indeed, as stated in Chapter XLI, several injections of neoarsphenamin may be required for producing the latter result because there are fewer spirochetes in the tissues, as indicated by the preceding negative reactions.

Spinal fluid changes in the nature of an increase of cells and protein, as well as of the Wassermann and colloidal gold reactions, have also been observed, due in part to the excitation of spirochetic activity.

As I shall discuss later, it is very important to follow up cases of these kinds with active treatment; merely to provoke a Wassermann reaction for diagnosis may arouse a latent infection with progression of the disease unless active treatment is continued.

Pathology of Jarisch-Herxheimer Reactions.—Very little can be written of the pathological changes occurring in the lesions of Jarisch-Herxheimer reactions, because of the scarcity of histological examinations and reports. Apparently there is an acute exacerbation of the inflammatory phenomena with acute hyperemia, serous exudation and an increase of infiltration of the tissues with small lymphocytes and plasma cells. The whole process including that of coagulation and liquefaction necrosis appears to be intensified. Perivascular infiltration is especially prominent, as likewise changes in the vessel walls tending to obliteration of the channels. The axis cylinders of nerves may be swollen and tortuous accompanied by infiltration of the sheaths with lymphocytes and plasma cells. The cells of an organ as of the liver and heart, may show albuminous or fatty degeneration as if subjected to the influence of an increased amount of toxic substances from the spirochetes, the drug or both.

Etiology and Nature of Jarisch-Herxheimer Reactions.—Many theories have been advanced to explain the mechanism of the Jarisch-Herxheimer reaction. The one mostly in favor and advanced by Wechselmann,[5] Ehrlich, Gennerich and others is that the reaction is due to an excitation of spirochetic activity in which the parasites increase in numbers and virulence along with the production of toxic substances in the nature of endotoxins from the destruction and disintegration of the parasites by the drug administered. Herxheimer and Kraus believed that the reaction was due to a hypersensitiveness of the cells in syphilitic foci to mercury, but of course this view was expressed before the discovery of *Spirochæta pallida* by Schaudinn.

The reaction is undoubtedly due in part, at least, to spirochetic activity. The pathological tissue changes, the finding of increased numbers of spirochetes by dark-field microscope examination of open lesions and the increase in degree of complement fixation indicate that this occurs, although there is little or no experimental support for the theory that the reaction is due to the liberation of endotoxins. The latter is merely a reasonable theory based upon analogy to the bacterial endotoxins.

But I doubt if this is the complete explanation of the mechanism. Hesse[6] and others, for example, have described reactions in non-syphilitic individuals with cutaneous eruptions similar to the Jarisch-Herxheimer reactions following the administration of arsphenamin in which the rôle of spirochetes could be excluded. It is well known that arsenic is vasculotoxic and by direct action upon the endothelium of capillaries or possibly by an effect upon the vasomotor center tends to produce vasodilatation which would naturally be particularly prominent and, indeed, may only occur, in areas of acute or

subacute inflammation. Furthermore, the spirochetes themselves produce vascular injuries and it may be that the Jarisch-Herxheimer reaction is a dual process due to the combined effects of both spirochetes and arsenic upon capillaries, but in which the rôle of spirochetes is of particular importance since the reactions do not tend to recur as the organisms disappear.

But is the Jarisch-Herxheimer reaction of this dual character in the case of mercury and bismuth? Neither of these drugs possess the same degree of vasculotoxic action of arsenic, but yet both, and particularly mercury, excite hyperemia and serous exudation in the tissues by their local irritant and corrosive action. Therefore, the presence of mercury or bismuth in the local lesions of syphilis may exaggerate the inflammatory phenomena of syphilis and I am of the opinion, as expressed in preceding chapters, that mercury tends to localize in syphilitic tissues by a process of specific organotropism or selective affinity for syphilitic exudates and *Spirochæta pallida* which we do not well understand at present. As previously stated, Herxheimer and Kraus thought that syphilitic tissues were hypersensitive to mercury; rather I believe there is this increased tendency for mercury (and possibly bismuth) to locate in syphilized tissues where they gradually reach a joint of concentration in which spirocheticidal effects are produced even though the drugs are not present in the blood or other tissues sufficient for producing toxic reactions. In addition to these local effects of mercury in syphilitic foci, there is the more important phase of exaggerated spirochetic activity, as in the reactions following the administration of arsphenamin and neoarsphenamin, and, as the parasites disappear, so does the tendency for Herxheimer reactions. But mercury and bismuth are far less likely to produce the reaction, because these drugs are absorbed more slowly and in small doses may bring about the destruction of the spirochetes without the production of Jarisch Herxheimer reactions at all.

The Jarisch-Herxheimer reaction, in other words, is analogous to the focal reactions of tuberculosis produced by tuberculin or focal reactions of other bacterial origin produced by the injection of autogenous vaccines. In these instances, the reactions are considered allergic in character, but the Jarisch-Herxheimer reactions are not because they occur in individuals who have never before taken the drugs and who present no evidences of natural allergy to them. But in both classes there is this increased affinity between the parasites and their destroyers; in the case of bacterial diseases, between the proteins of the vaccine and the antibodies engendered by them; in the case of syphilis, between the spirochetes and spirocheticidal drugs. Just as focal reactions in tuberculosis and other infections may be produced by large injections of sterile milk and other non-specific agents, because in the lesions the blood-vessels are more subject to dilatation, so likewise in non-syphilitic lesions may the vasculotoxic effects of arsenic be exaggerated, explaining the occurrence of Herxheimer-like reactions in non-syphilitic diseases treated with arsphenamin and its substitutes.

Diagnosis of Jarisch-Herxheimer Reactions.—The diagnosis of these reactions is not usually difficult since they bear an intimate time relation to the administration of anti-syphilitic drugs occurring in a matter of a few hours to a day or two after the injection of arsphenamin or neoarsphenamin or in from one to several days after the intramuscular injection of mercury or bismuth. Jaundice is particularly difficult of differential diagnosis because in hepatic Herxheimer reactions this sign develops slowly and in any given case may be due to syphilis and not to the drug.

Prevention of Jarisch-Herxheimer Reactions.—I have discussed this subject with more detail in Chapter LXXIV, because it is one of considerable

practical importance when the treatment of syphilis is instituted in the tertiary stage and particularly if the cranial nerves (the oculomotor, optic, auditory and facial nerves) are involved or if symptoms of tabes are present. Here it may be stated, however, that these reactions may be generally avoided by instituting treatment with small doses of the chosen remedy, that is, with 0.1 to 0.2 gm. of arsphenamin or neoarsphenamin instead of the usual 0.6 to 0.9 gm. doses or by commencing treatment with the administration of small doses of mercury before more vigorous therapy is attempted.

Treatment of Jarisch-Herxheimer Reactions.—Specific treatment is generally demanded and consists of the administration of more arsphenamin, neoarsphenamin or other antiluetic drug. It is true that this therapy demands courage on the part of both physician and patient, and it is sometimes particularly difficult for the latter to submit to another injection of a drug that brought on his pain and disability. But whenever the physician is reasonably sure that the reaction has occurred and is not a direct toxic action of the drug, it is proper and almost imperative in some cases to continue with specific medication. In such instances, however, it is my custom to give smaller doses but at more frequent intervals.

In addition it may be necessary to afford relief from pain in tabes by the administration of morphin. The headache of syphilitic meningitis may require rest in bed. Spinal puncture sometimes affords relief as likewise the internal administration of large amounts of sodium chlorid as described in Chapter LI for the treatment of hemorrhagic encephalitis.

Syphilitic Recurrences.—Syphilitic recurrences generally occur several weeks after the cessation of active antisyphilitic treatment. For this reason they are not usually confused with Jarisch-Herxheimer reactions, but may be, and frequently are, confused with the late toxic effects of the drug.

Syphilitic recurrences are due to a resumption of activity of spirochetes escaping destruction. It is possible, however, and indeed, probable, that these effects are aided by the production of localized areas of reduced tissue resistance by the toxic action of the drugs administered. There is, therefore, frequently the possibility of a dual etiological character to lesions of this kind and particularly in syphilis of the liver. Furthermore, insufficient treatment tends to depress immunological resistance in syphilis which may be an additional factor and especially in the recurrences of early syphilis. As mentioned in previous chapters, the development of immunity in syphilis depends upon the presence of living spirochetes in the tissues. This does not mean that we should leave syphilis untreated because the acquired immunity is probably never sufficient for effecting a cure, but it does mean that when we treat syphilis we should keep on treating until the spirochetes are destroyed. In other words, insufficient and inadequate treatment depresses immunity without removing the spirochetes and the latter may then spring into activity with greater dissemination as soon as specific treatment ceases; in this manner the question of kind and amount of treatment bears an additional and important relation to syphilitic recurrences.

Syphilitic recurrences of the nervous system are generally designated as "neuro-recurrences" or "neuro-recidives" and, are discussed more fully in Chapter LI in relation to neuritis sometimes produced by the organic arsenical compounds and with which they are particularly likely to be confused; recurrences in the liver are designated as "hepato-recurrences" or "hepato-recidives" and are discussed in the same chapter. Recurrences after courses of mercury and bismuth are more apt to be true syphilitic recurrences, because these drugs are much less likely to produce neuritis, hepatitis and allied lesions due to the toxicity of the drugs.

Syphilitic recurrences are more likely to develop in the tertiary stage of the disease when treatment is started and then interrupted. Therefore, in cases yielding positive "provocative" Wassermann reactions, treatment should always follow. Recurrences are likely to occur in the oculomotor, optic, auditory and other cranial nerves due to syphilitic activity and the laying down of increased numbers of lymphocytes and plasma cells in the sheaths; in cases of this kind, there is usually no suspicion of toxic effects on the part of the drug administered if it happens to be arsphenamin, neoarsphenamin, mercury or bismuth, because these compounds do not usually produce toxic amblyopias in case the optic nerves are involved or similar lesions of other nerves.

But if one of the pentavalent organic arsenical compounds have been administered, and particularly atoxyl or tryparsamid, due care must be exercised in diagnosis because these compounds are especially likely to produce toxic amblyopias, as discussed in Chapter XLVI.

The *treatment* of syphilitic recurrences demands the administration of antiluetic drugs providing one can be sure that the condition is a true recurrence due to spirochetic activity rather than due to some toxic effect of the drug. In case of doubt, it is well to resume treatment with some other agent. For example, in cases of recurrences of the optic and auditory nerves or other parts of the nervous system as well as in liver recurrences, following courses of arsphenamin or neoarsphenamin, it is my custom to institute treatment with a course of injections of potassium tartro-bismuthate or of mercury by inunction. In this manner, one escapes making the mistake of possibly adding to the toxic effects already produced by arsenic.

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PART IX

VARIOUS METHODS FOR THE ADMINISTRATION OF ANTISYPHILITIC MEDICAMENTS

CHAPTER LIX

TECHNIC OF VENOUS PUNCTURE AND OF GRAVITY AND SYRINGE METHODS FOR INTRAVENOUS INJECTIONS

The technic of intravenous injections has undergone many improvements and refinements during the past fifteen years since the introduction of arsphenamin and neoarsphenamin in the treatment of syphilis. Prior to that time it was the general custom to incise the skin and expose a vein, but this practice is not now resorted to except in unusually difficult cases. Many different kinds of apparatus and needles have been described, but the simplest are generally very satisfactory when the principles are understood and experience has been gained in the technic of venous puncture and intravenous infusions. As in all other fields of surgical practice, experience and practice are required for the highest proficiency in intravenous therapy and those lacking in experience should first work under the observation and with the assistance of one skilled in the methods. Venous puncture and intravenous therapy form a very important part of the modern treatment of syphilis and it is necessary for those physicians undertaking the treatment of this disease to perfect themselves in this work so that not only one, but a series of intravenous injections, may be given patients without scarring, undue pain and disability.

In this chapter I shall explain with considerable detail the methods which I have employed for several years with uniform success; these methods are applicable not only for the administration of the organic arsenicals but for mercury, the iodids and whatever other drugs may be administered by intravenous infusion.

Importance of Good Technic.—In no other field of intravenous therapy is it as necessary to possess as much skill and nicety in technic, as in the treatment of syphilis. If only one or two injections of arsphenamin or its substitutes were sufficient, most individuals would be willing to withstand almost anything in the way of pain and scarring, but such is not the case. It is now well established that a series of injections are required and it is therefore necessary to give these by the most painless methods free of "tell-tale" marks. The dreadful pain and disability resulting from large accidental perivascular injections of arsphenamin and neoarsphenamin imposes upon the physician a heavy responsibility in making the injections. There should be no compromise with make-shift apparatus, rusty and dull needles, defective syringes and short-cut hasty methods. The best is not too good and good work should never be sacrificed for mere speed.

It is especially necessary to avoid incising the skin and exposing veins on account of the resulting scars. In this day the laity are becoming quite well informed on the usual significance of scars about the elbows and the patient should be spared them whenever possible. This is especially true of women but of no lesser importance to men and especially those required to work with bared arms.

Choice of Veins.—In the great majority of cases the veins of the forearm and especially those about the elbow are employed as the median cephalic, median basilic, common ulnar and radial veins (Fig. 31). The median, ulnar and cephalic veins are also employed; indeed, any prominent, *well supported* vein may be used, but the median basilic is probably the least desirable for the inexperienced because of the proximity of the internal cutaneous nerve and because it is separated from the brachial artery by the biceps fascia which may be pierced by clumsy technic.

The most prominent vein is not always the easiest to enter because it may roll under the needle; under such circumstances it is especially advisable to use a short and very sharp needle. The best veins are those which are full but well supported by the subcutaneous tissues to prevent rolling and dimpling while the needle is being introduced.

In some cases the veins on the back of the hand may be employed; however, never by choice but only by necessity, since they are small, very thin, easily pierced and likely to give subcutaneous hemorrhages. The same is true of veins about the ankle. When these veins are employed the needle must be short, very sharp and not larger than gauge No. 20 or 22.

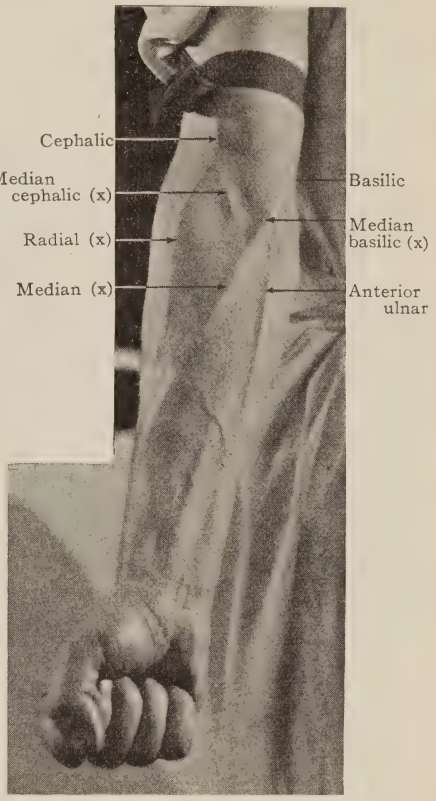

FIG. 31.—CHOICE OF VEINS FOR INTRAVENOUS INJECTIONS AND SECURING BLOOD.

The sites of choice are marked with an (x); it is always advisable to avoid punctures near the crease of the elbow in order to avoid subsequent discomfort.

Children from one to six years are the most difficult of all to inject intravenously because their veins are not only very small and usually well covered, but because they usually struggle during the operation. If the child can be induced to keep quiet one can frequently inject them with a small needle (No. 22) and syringe by way of a vein about the elbow; otherwise one may use the superficial temporal or the external jugular veins. Naturally one hesitates to use the latter because of the proximity of large vessels, but in practice the method is quite simple and safe.

In infants under a year the superior longitudinal sinus may be employed, but never as a matter of choice for the injection of arsphenamin or neoarsphen-

amin, because of the serious reactions and fatalities which have followed accidental injections into the substance of the brain from transfixion of the sinus. I believe that one of the external jugular veins is always to be preferred and if these cannot be used, intramuscular or even intraperitoneal injections may be resorted to. The intravenous injection of arsphenamin or one of its substitutes is rarely required as a life saving procedure as may be blood transfusion or the intravenous infusion of glucose or sodium bicarbonate, and for this reason I do not approve of jeopardizing the life of an infant by sinus injections of antisyphilitic drugs.

Position of the Patient.—In drawing blood for the Wassermann test and in giving intravenous injections of concentrated solutions of neoarsphenamin and sulpharsphenamin, the patient may sit upright alongside of a table with the arm extended and a support under the elbow (Fig. 32); if, however, the patient is nervous and feels that he or she may become ill or faint, it is better

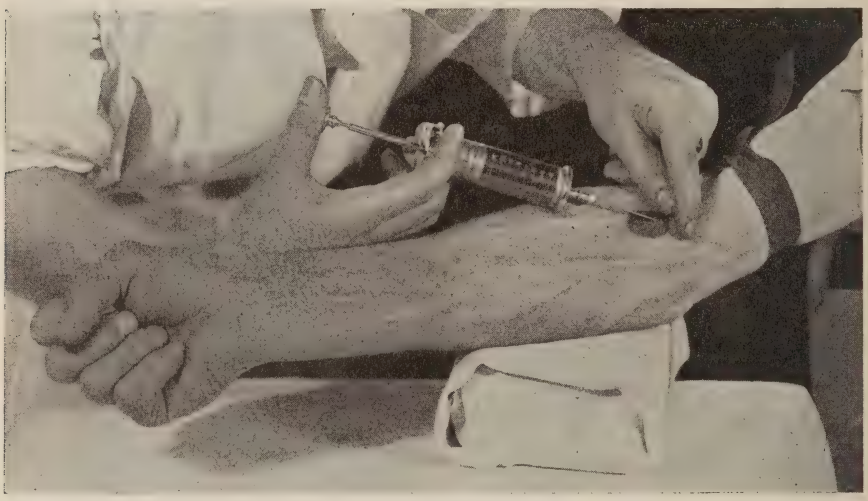

FIG. 32.—SITTING POSITION FOR INTRAVENOUS INJECTION OR WITHDRAWAL OF BLOOD.
The elbow is supported in order to secure maximum extension of the arm; the hand is rapidly opened and closed and finally clenched before puncture of the vein in order to render it full and prominent.

to have the patient lying on a table with the arm extended. Whatever the position may be, the patient should be as comfortable as possible and especially the arm, if the position must be maintained for five minutes or longer; otherwise movements may occur tending to displace the needle. The latter position is always advisable for gravity injections of arsphenamin and neoarsphenamin. In the prone position the patient is not as well able to watch the manipulations and it is always the position of choice for intravenous infusions by both syringe and gravity methods because it allays nervousness, enables the patient to assume a comfortable position and prevents accidents due to sudden faintness and nausea.

Application of the Tourniquet.—The practical importance of a proper tourniquet is not to be overlooked. It should be at least an inch wide in order to produce the minimum of discomfort and should be applied in such manner as may be released instantly and with no commotion in order not to disturb the needle and injection.

In many instances a tourniquet may be improvised by twisting a towel, a piece of bandage or the shirt sleeve and held by an assistant or the patient, but I regard these procedures as inferior to a piece of ordinary garter elastic or a strip of rubber sheeting held by a slip knot. Sometimes an assistant or even the patient himself may firmly grasp the arm and distend the vein sufficiently. The ordinary surgical tourniquet is too clumsy and generally unsatisfactory; the tourniquet of rubber tubing held by a hemostat is only a makeshift, apt to wrinkle and pinch the skin. The cuff of a sphymanometer makes a most excellent tourniquet, being broad and comfortable and readily applied and released.

My favorite tourniquet is 15 inches of good garter elastic about an inch wide applied with one firm turn and held by a slip knot (Fig. 33) which is

FIG. 33.—SHOWING THE DETAILS OF A SATISFACTORY SLIP KNOT TOURNIQUET OF ORDINARY GARTER ELASTIC.

released instantly by a slight pull without disturbance. The tourniquet should be applied flat without wrinkling or pinching the skin.

After applying the tourniquet the patient should open and close the hand vigorously for a few seconds and keep the hand clenched during the introduction of the needle; this aids in distending the superficial veins.

The tourniquet should not be applied too soon; all should be in readiness, as the most comfortable tourniquet becomes very uncomfortable as venous stasis is maintained over several minutes.

If the veins are small and deep, as in the case of obese women, it is sometimes helpful to apply an additional tourniquet from the wrist upward to the middle of the forearm (Fig. 34) after a tourniquet has been applied above the elbow. Under such circumstances it is also sometimes helpful to have the patient first bathe the hand and arm in hot water followed by

brisk rubbing, immediate application of a tourniquet and massage of the arm upward.

If the veins on the back of the hand are employed, it is well to first have the patient soak the hand in hot water for several minutes followed by brisk drying and the application of a tourniquet about the wrist.

If the external jugular vein is employed, an assistant is required to make compression of the neck just above the clavicle as shown in Fig. 43; an assistant may also distend a vein of the scalp by digital compression.

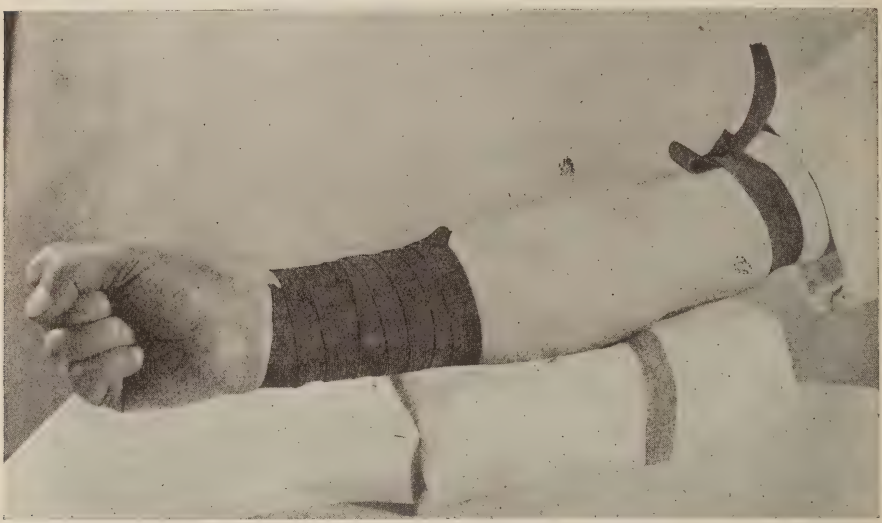

FIG. 34.—A DOUBLE TOURNIQUET SOMETIMES OF VALUE IN OBESE INDIVIDUALS OR THOSE WITH SMALL AND DIFFICULT VEINS.

Preparation of the Skin.—For simple venous puncture it is sufficient to prepare the skin by cleansing with 1:1000 mercurophen or bichlorid of mercury in alcohol; tincture of iodin (5 per cent.) or a solution of picric acid (5 per cent.) may be applied, but if so, they should be wiped away with alcohol so as not to hide the vein.

If it is necessary to incise the skin, there should be thorough preliminary cleansing with soap and water, following by iodin and a final cleansing with alcohol in order to guard against infection of the skin to prevent scar formation as much as possible.

Technic of Venous Puncture.—When all is in readiness, the tourniquet applied, vein selected and skin prepared the needle may be entered into the vein. If the patient is nervous and needle-shy, the site of puncture may be briefly sprayed with ethyl chlorid or a drop or two of sterile 1 per cent. solution of butyn may be injected *into the skin* with a 1 c.c. syringe fitted with a No. 26 needle. The latter, however, adds to the difficulties, may interfere with the vein puncture and great care must be exercised against puncturing the vein as perivascular bleeding will occur until the tourniquet is released, withthe production of a hematoma which interferes with subsequent puncture at this site. As a general rule no anesthesia is required for No. 22 needles and especially if a short sharp needle is employed; with No. 19 or larger needles, one or the other of these anesthetics may be required for nervous patients but this is exceptional rather than routine practice.

The vein should be steadied with the thumb and index finger; some physicians prefer to slightly stretch the skin over the vein to steady it although this tends to flatten the vein and increase the chances of transfixion. Various clamps have been devised for grasping and steadying the vein

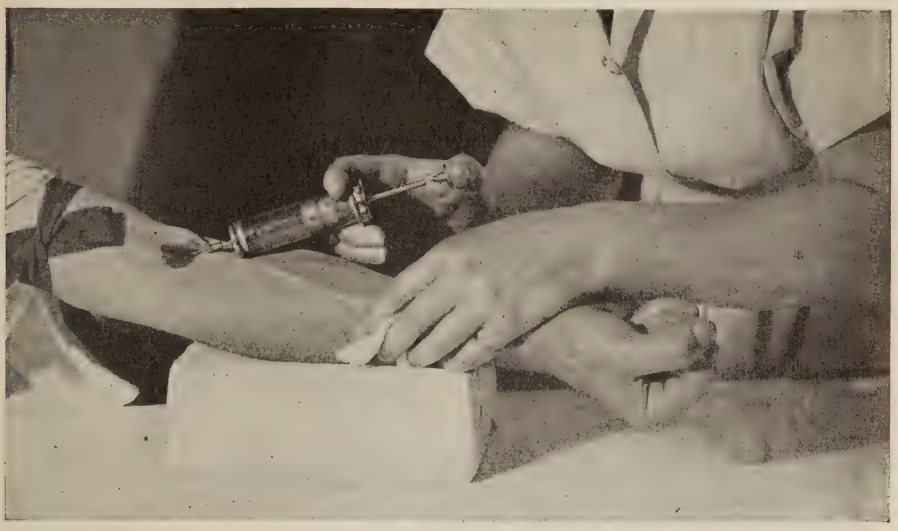

FIG. 35.—CORRECT ANGLE FOR HOLDING A SYRINGE FOR ENTERING A VEIN.
The syringe is held at an acute angle to the arm with the needle bevel upward.

FIG. 36.—INCORRECT ANGLE FOR HOLDING A SYRINGE FOR ENTERING A VEIN.
The syringe is being held at such an angle as increases the danger of transfixing the vein with the needle; the arm is poorly supported and the veins poorly distended.

through the skin but there is no better means than the finger and thumb of the disengaged hand.

The needle should be held at an acute angle to the arm (Fig. 35); it is a mistake to attempt entering the vein at a right angle because of the danger of passing through the vessel (Fig. 36). It is also good practice to puncture

the vein obliquely, that is, with the needle directed to the side of the vein rather than directly over its top and especially if the vessel is very prominent, sclerotic and freely movable. When an attempt is made to puncture directly over the vein the needle sometimes slips over its top and passes into the perivascular tissues. When, however, the vein is not prominent but is felt full and elastic in the subcutaneous tissues, one may enter the needle directly over the vein as the vessel is well supported and unable to roll.

When blood is being withdrawn or an injection is to be given with a syringe the needle should always be firmly attached, as the syringe affords an excellent handle for the needle. If an injection is to be given by gravity the

FIG. 37.—"FLASH" OF BLOOD SHOWING THAT THE NEEDLE IS WITHIN THE LUMEN OF THE VEIN.

unattached needle may be entered into the vein and the tubing attached when a free flow of blood is observed. If, however, a gravity injection is being given by a short Record or Luer needle, a sterile 5 or 10 c.c. syringe carrying saline solution may be loosely attached as a handle, the syringe being gently detached when a flow of blood into the syringe has been observed, and the tubing attached.

Evidence that the needle has entered the vein will be found by the fact that there is a peculiar "give-away" sensation to experienced fingers and blood appears in a *free* flow either from the needle or into the syringe as the piston is slightly withdrawn. The "flash" of blood (Fig. 37) should be free; if only a few drops appear it is almost sure that the lumen of the needle is not entirely in the vein. Sometimes a scanty flow of blood is due to partial

blocking of the vein but this is not at all likely if the needle has been tested before use as it should be; it is bad practice to pass a wire into the lumen while the needle is in place as an embolus may be thrown into the blood stream. The blood of tuberculous individuals having a high viscosity may not flow easily according to some physicians, but as a general rule no difficulties are encountered.

If the blood does not flow one may depress or rotate the needle, push it in a little further or withdraw it very slightly and enter it in a new direction, but if perivascular bleeding occurs, indicating that the vein has been pierced, it is well to loosen the tourniquet and withdraw the needle. After a few minutes the tourniquet may be re-applied and a fresh attempt made. Likewise if the needle is withdrawn because a "flash" of blood has not been observed, and bleeding follows from the wound, the tourniquet should be released at once and gentle pressure made over the puncture for a few minutes to prevent perivascular bleeding. The tourniquet may now be reapplied for a fresh attempt but it is always unwise to endeavor to puncture the vein at the same site and especially if a hematoma has been produced.

When blood is being withdrawn for the Wassermann test the tourniquet should not be released until the required amount is secured. *But the tourniquet should be always released before the needle is withdrawn in order to prevent subcutaneous and perivascular hemorrhage.* When an injection is to be given the tourniquet should be released, but with syringe injections this is not always necessary and should never be attempted at all if the arm must be moved with danger of displacing the needle. With slow injections the vein does not usually "balloon" and the tourniquet aids in bringing about slow admixture of blood and solution of drug. With gravity injections, however, the tourniquet should be always released to prevent "ballooning" of the vein and perivascular bleeding.

Methods for Obtaining Blood for the Wassermann Test. *With the Keidel Tube.*—One of the most satisfactory methods of obtaining blood from a vein quickly, aseptically and with the least pain is by means of the Keidel tube (Fig. 38). As made at present by Mr. Comer for the Steele Glass Company of Philadelphia, this very useful apparatus is available with a glass window permitting one to see a flow of blood, and thereby indicating that the vein has been entered, before the neck of the ampule is broken. The apparatus is dispensed sterilized and ready for use as follows:

1. The patient may sit alongside of a table or desk with the arm extended and elbow supported.

2. The tourniquet is applied above the elbow and the patient requested to open and close the hand vigorously.

3. The skin is wiped with cotton and alcohol.

4. The wire is removed and the hub of the needle grasped (avoiding contamination of the shaft) between the thumb and first finger (Fig. 38) and entered through the skin into the vein at an acute angle to the arm. It is a mistake to enter the needle at an obtuse or right angle because it may be passed through the vein.

5. As the vein is entered there is a peculiar "give-away" sensation readily appreciated by experience; if the "window" type of apparatus is employed a flow of blood is seen.

6. The long stem of the ampule in the rubber tubing is now crushed with the fingers or by a hemostat (Fig. 39) and the ampule filled with blood by suction owing to the release of the vacuum.

7. If blood does not flow the ampule may be rotated, in case the lumen of the needle is in contact with the inner wall of the vein. If it is apparent that

the needle is not in proper position it may be partly withdrawn and another attempt made, due care being taken not to withdraw the eye of the needle

FIG. 38.—SECURING BLOOD WITH THE COMER-KEIDEL TUBE; METHOD OF PASSING THE NEEDLE.

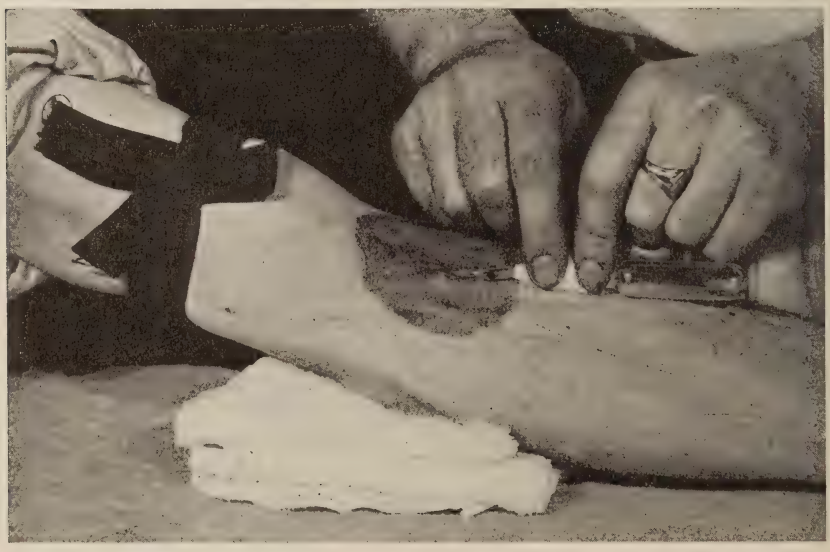

FIG. 39.—SECOND STEP IN SECURING BLOOD WITH THE COMER-KEIDEL TUBE. Blood is seen in the "window" indicating that the needle is in the lumen of the vein; the neck of the ampule is being broken to release the vacuum followed by suction of blood.

from beneath the skin, which permits the entrance of air, in case the neck of the ampule has been broken.

8. The tourniquet should be released before the needle is withdrawn to prevent subcutaneous hemorrhage.

9. The site of puncture is compressed for a few seconds and cleansed with alcohol; a dressing is not required.

10. If the blood is to be mailed it is necessary to bend down the rubber tubing and hold it in this position by a rubber band or tying with a piece of string to prevent leakage of blood or serum in transit.

With a Syringe.—Blood may be also obtained from a vein in the same manner by means of a sterilized 5 or 10 c.c. Luer or Record syringe fitted with a No. 20 to 22 needle; it is unnecessary to use a larger needle which inflicts unnecessary pain. The needle should be short and not over 1¼ inches; long needles interfere with the flow of blood and are more difficult to enter into a vein. The bevel must be short rather than long but not too

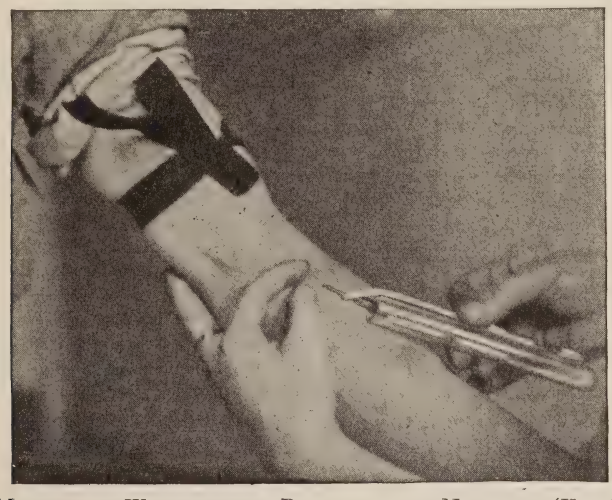

FIG. 40.—METHOD OF WITHDRAWING BLOOD WITH A NEEDLE. (Keen's Surgery.)

stumpy; it is almost imperative to use a sharp and rust-free needle to conduct the operation with the minimum of pain and without leaving a stained puncture site in the skin.

After securing the blood the tourniquet should be released before the needle is withdrawn; the blood should be emptied into a sterile test tube or better, into a small sterile vial tightly stoppered if the specimen is to be mailed. For this purpose the vial should not be of more than 10 c.c. capacity in order to reduce to a minimum the agitation and consequent hemolysis of corpuscles in transit.

The test-tube or vial need not necessarily be sterile if the blood is to be tested within forty-eight hours but if there is a longer interval, the container should be sterilized beforehand by dry heat or it may be boiled for a few minutes, drained and cooled just before use. It is imperative, however, under all circumstances, that the container shall be *chemically clean* and especially free of acids and alkalies which are hemolytic and anticomplementary.

With a Needle.—Blood may be secured from a vein by merely entering a sufficiently large sterilized needle and allowing it to flow into a prepared test-tube or vial. For this purpose the needle should be gauge 18 to 20 since suction is not applied, and the shaft should not be too long as otherwise

coagulation may occur. It is of advantage to grasp the needle with a hemostat as shown in Fig. 40. This method, however, is apt to be "messy" and is inferior to the Keidel tube and syringe methods.

By Puncture of a Finger.—From 3 to 5 c.c. of blood is readily obtained from a finger and is perfectly acceptable for the Wassermann test. In obese individuals with difficult veins or in nervous individuals who are almost sure to faint if venous puncture is attempted, this method is very valuable although it requires a little more time and patience. The technic is as follows:

1. The hand should be warm. If cold and clammy have the patient immerse the hand in hot water for a few minutes followed by brisk drying.

FIG. 41.—METHOD OF PRICKING A FINGER.

The patient's finger is grasped firmly and lanced with a Daland lancet *across the folds of the skin.* When lanced parallel with the skin-folds the wound is likely to close before sufficient blood is secured.

2. Select the middle or ring finger; cleanse with alcohol and dry.

3. Cleanse the broad blade of a Daland blood lancet with alcohol and puncture deeply across the lines of the tip of the finger (Fig. 41) with a sharp broad lancet; there is very little pain.

4. Massage the blood into a *small* test-tube or vial (Fig. 42); it is a mistake to use a large receptacle as blood is wasted on its walls.

5. Cleanse the finger and apply compression for a few seconds. A dressing is not ordinarily required unless bleeding continues.

Securing Blood from Infants and Children.—A few cubic centimeters of blood may be readily obtained from infants by puncturing one of the great toes with a Daland lancet and massaging the blood into a small test-tube, as described above for securing blood by puncture of a finger.

In the case of children from 1 to 6 years sufficient blood may be obtained by puncture of one or several fingers. In children over 6 years of age, a vein may be entered and blood drawn with a Keidel tube or syringe as above described.

Blood may be obtained with a syringe from one of the external jugular veins as shown in Fig. 43 or from a temporal vein.

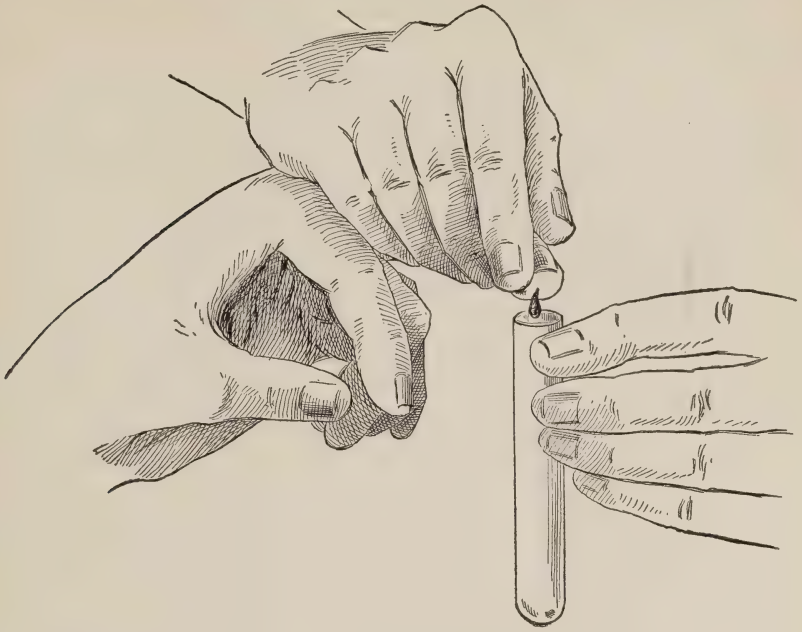

FIG. 42.—METHOD OF SECURING A SMALL AMOUNT OF HUMAN BLOOD FROM A FINGER.
By pricking the finger deeply *across* the lines of the skin with a *broad* lancet two or more cubic centimeters of blood are easily collected in a small test-tube. Do not use a large tube, as blood may be lost on the sides of the tube.

FIG. 43.—METHOD OF OBTAINING BLOOD FROM AN EXTERNAL JUGULAR VEIN.
The patient is a child six years of age; shows the position of the patient and manner of distendng the vein by pressure above the clavicle. A 5 c.c. Record syringe fitted with a No. 20 needle is being used for the withdrawal of blood. The distended vein is painted with tincture of iodin to indicate the position. (Keen's Surgery.)

Blood may be secured from infants under one year of age by puncture of the superior longitudinal sinus (Figs. 44 and 45) as follows:

1. The infant is wrapped in a blanket and the head is steadied by an assistant (Fig. 46).

2. The puncture is made on the median line of the posterior angle of the anterior fontanelle.

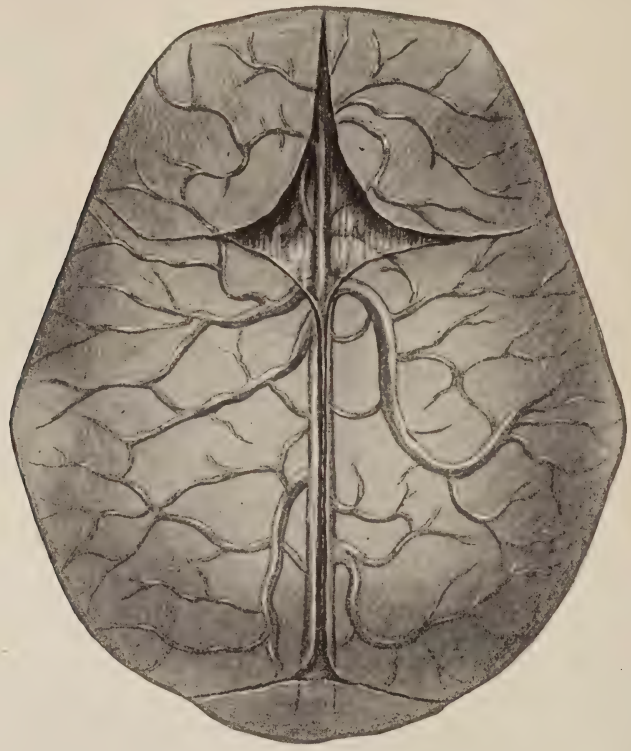

FIG. 44.—THE ANTERIOR FONTANEL AND RELATIONS AND SIZE OF THE SUPERIOR LONGITUDINAL SINUS IN A NEWBORN INFANT. (Keen's Surgery).

FIG. 45.—A CROSS-SECTION OF THE HEAD OF A NEWBORN INFANT THROUGH THE POSTERIOR ANGLE OF THE ANTERIOR FONTANEL TO SHOW THE SIZE AND SHAPE OF THE SUPERIOR LONGITUDINAL SINUS. (Keen's Surgery.)

3. The skin is carefully cleansed. The needle, gauge No. 18, with a short bevel, sterilized and attached to a sterile 5 c.c. Record or Luer syringe is

passed inward at a right angle for a distance of about 4 mm. and suction made; if blood does not flow the needle should be passed about 2 mm. further, which suffices for the majority of children up to fifteen months of age.

4. At least 3 to 5 c.c. of blood may be safely withdrawn and discharged into a vial. The puncture site is then cleansed and may be sealed with a touch of collodion.

FIG. 46.—METHOD OF OBTAINING BLOOD AND GIVING AN INTRAVENOUS INJECTION BY WAY OF THE SUPERIOR LONGITUDINAL SINUS.

The child is six months of age; the shape and size of the anterior fontanel have been outlined; a 5 c.c. syringe fitted with No. 18 needle is being employed, the needle having been entered for about 6 millimeters in the median line at the posterior angle and perpendicular to the sinus.

Methods for Obtaining Blood in Connection with Intravenous Infusions.—It is a very easy matter to obtain blood just before an intravenous injection is to be given. When administering neoarsphenamin or sulpharsphenamin with a Record or Luer syringe one may have the solution ready for injection in one syringe, but enter the needle attached to a second syringe, remove blood, *loosen the tourniquet*, deftly detach this syringe, attach the second syringe containing the solution and inject. As a general rule, coagulation will not occur before it is possible to empty the blood into a vial.

If the infusion is by gravity the same plan may be followed by entering the vein and collecting blood in a tube before attaching the tubing, releasing the tourniquet, attaching the tubing and infusing the solution. For this method however, it may be necessary to have assistance.

Not infrequently I pass the needle of a Keidel tube and fill the bulb with blood; then leaving the tube supported on the arm and before releasing the tourniquet, the needle attached to the syringe carrying the solution is entered *above*, the tourniquet released and the injection slowly given when both needles are withdrawn simultaneously. This means two punctures but the needles are small, the pain is almost neglible and both procedures are carried through without the loss of a single drop of blood.

Technic of Intravenous Infusion by Gravity Methods. *Apparatus Required.*—During the past twelve years many different kinds of apparatus and needles have been described and advocated for the intravenous infusion of dilute solutions of arsphenamin and neoarsphenamin. The simplest sort

of apparatus, however, will suffice when the technical principles are understood. That shown in Fig. 47 is sufficient if assistance is available; that shown in Fig. 48, is very satisfactory when the injections are to be given without assistance. One of the cylinders carries saline solution for the first and last part of the infusion; the second cylinder carries the solution of arsphenamin or neoarsphenamin and both are connected to the needle by a Y-glass tube or a Morton three way cock attached to six inches of rubber tubing carrying a glass window or glass adapter. The cylinders should be of at least 200 and preferably 300 c.c. capacity plainly calibrated from above downward.

FIG. 47.—METHOD OF MAKING INTRAVENOUS INJECTION BY GRAVITY.

This method is suitable for the intravenous administration of dilute solutions of arsphenamin, neoarsphenamin, sulpharsphenamin, etc. The needle has been entered into a prominent vein (indicated by a flow of blood); the tubing has been attached by means of a metal adapter which fits the needle easily and snugly; the tourniquet has been loosened and the injection is being given.

Proper care of the needles is important. They should be cleansed immediately after use, and precautions taken to prevent rust. Just before sterilization, the point should be freshened on a stone, if necessary. The bevel must not be too long, because of the danger of piercing the vein through and through or leaving the opening of the needle partly within and partly outside of the vein; neither should it be too short or stumpy. The first essential for success and a satisfied patient is the right sort of needle, bright, clean and sharp.

A piece of glass tubing, 6 mm. in diameter, should be inserted in the rubber tubing so as to be a few inches from the needle. A bulb in the tubing of ¾ inch diameter outside as shown in Fig. 49, is better than straight tubing as it permits bubbles of air to collect in the bulb, should any be present in the tubing.

The adapter at the end of the tubing must fit the needle snugly, but not too tightly, so that it may be attached or detached with the minimum of disturbance. Pinch cocks (Mohr's) are satisfactory for cutting off the flow of solutions.

Preparation of Apparatus.—As described in Chapter XLVI, toxic reactions may be produced by new rubber tubing used for the first time. It should be

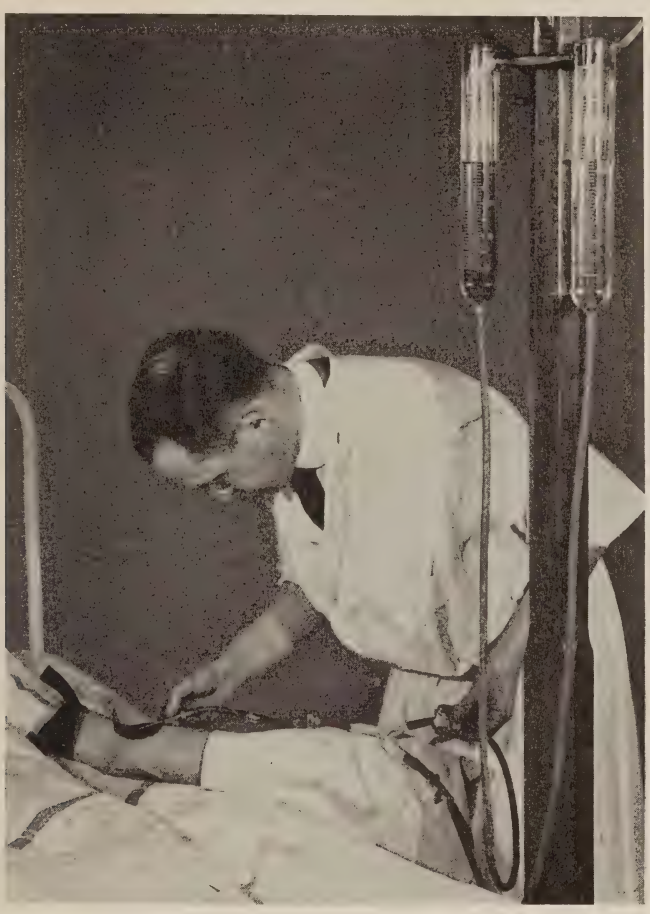

FIG. 48.—METHOD OF MAKING INTRAVENOUS INJECTION BY GRAVITY.

One cylinder carries sterile saline solution; the second carries the solution of arsphenamin or other drug to be injected. Both are connected to the needle by a Y-glass tube attached to six inches of rubber tubing carrying a glass window; pinch cocks are provided or a three way cock may be used.

soaked in normal sodium hydroxid solution (4 per cent.) for at least six hours and thoroughly rinsed in running water before sterilization.

All the apparatus, including cylinders, tubing and needles, may be sterilized by boiling for at least five minutes and cooled. Sodium carbonate should not be added to the water. Just before use the cylinders and tubing should be flushed out with sterile saline or sterile distilled water.

Everything should be in readiness, the solution of arsphenamin or neoarsphenamin being ready for infusion before the tourniquet is applied and the

needle is entered into a vein. The methods for preparing solutions are described in Chapter LXII.

Technic of Injection with One Cylinder.—The method is as follows:

1. Assemble the apparatus complete; pour in sterile saline solution (preferably at about 38°C.) and expel all air. Leave at least 30 c.c. in the cylinder; pinch off. An assistant should be available to hold the cylinder about 3 feet above the vein of the patient or the cylinder may be fastened to some support. An assistant may be required, however, to pour in the solution of arsphenamin or neoarsphenamin at the right time followed finally by more saline solution.

2. Adjust a tourniquet and prepare the skin.

3. Enter the detached needle and make sure that it is sufficiently far in the lumen of the vein with a free flow of blood. If the blood appears only in drops with a gauge 19 needle the lumen is partly obstructed.

FIG. 49.—GLASS BULB INSERTED IN RUBBER TUBING TO SERVE AS A WINDOW AND TO COLLECT BUBBLES OF AIR.

4. Deftly attach the adapter and release the pinch cock; now release the tourniquet. Watch the site of injection very carefully. There should be no pain and no swelling; the saline should flow freely.

5. An assistant should now pour the solution of arsphenamin or neoarsphenamin into the cylinder.

6. The rate of flow will be about right with tubing and a needle of the sizes advised; the flow should be even, slow and not exceeding 25 c.c. per minute. It is highly desirable, in sensitive patients, to wait a minute or two after injecting the first 10 to 20 c.c. before proceeding with the rest of the infusion.

7. As the solution reaches the 20 c.c. mark on the cylinder add about 30 c.c. more of saline and continue the infusion until the last is seen slowly passing through the glass window.

8. Pinch off the tube and quickly withdraw the needle. Cleanse the skin and seal the puncture with a bit of sterile cotton and a drop of flexible collodion.

Technic of Injection with Two Cylinders.—With this apparatus an assistant is not required and not a few patients appreciate strict privacy while undergoing treatment for syphilis.

1. In assembling the apparatus both cylinders are attached to a Y-glass tube with about 2½ feet of tubing; a pinch cock is attached to each just above the connections. The straight arm of the glass tube is then attached to about 6 inches of rubber tubing carrying a glass window and an adapter for the needle; the adapter may be of glass in which case a glass window is unnecessary. (Fig. 49.)

2. Saline solution is placed in *both* cylinders and the pinch cocks released until all air has been expelled. About 30 c.c. of saline is left in each cylinder; the arsphenamin or neoarsphenamin solution is poured in one.

3. When all is in readiness the tourniquet is applied, the skin prepared and the needle entered. When a free flow of blood is secured the adapter is deftly attached, the tourniquet released and the pinch cock released on the tubing connected with the cylinder carrying saline.

4. When it is observed that the saline flows freely with no pain or swelling about the site of puncture, the pinch cock is applied and the pinch cock connected with the tubing attached to the cylinder carrying arsphenamin solution is released.

5. The rate of flow will be about right with the tubing and needle of the sizes advised; the flow should be even and not over 25 c.c. per minute.

6. After the arsphenamin solution has disappeared from the cylinder the pinch cock should be applied and the cock to the saline carrying cylinder released for a few seconds to wash in the arsphenamin in the lower end of the tubing.

7. The tubing above the needle is now pinched, the needle quickly withdrawn, the skin cleansed and the puncture site sealed with a bit of cotton and collodion.

Technic of Intravenous Infusion by Syringe Methods. *Apparatus Required.*—Perfectly adjusted and smoothly working syringes are absolutely necessary. The Record and Luer syringes are particularly good; rubber plunger syringers are generally unsatisfactory not only because they are apt to work stiffly but because they are not easily sterilized.

My own preference is for the 20 c.c. size for routine injections of neoarsphenamin and sulpharsphenamin by way of the veins about the elbow and the 2 c.c. size for the injection of highly concentrated solutions by way of the veins on the back of the hand. A 10 c.c. size, however, can be used for both, utilizing the ampules of sterile distilled water furnished by the makers of the products, as well as for the injection of infants by way of the temporal or external jugular veins.

The needles should be gauge No. 22 with a short shaft; long shafted needles are more difficult to handle and should not be used. The needle should be bright and free of rust, very sharp and with a short bevel. It is important that the needle fit the syringe snugly so that there may be no leakage during the injection; the piston should also fit the barrel properly so that there may be no "back-firing" or sticking during the injection. It is well to assemble the syringe and needle and test it out with sterile water or saline before use. If the syringe has been used for a prior injection it should be thoroughly washed out free of all traces of blood and re-sterilized; otherwise the piston is apt to "stick."

Preparation of Apparatus.—The syringe and needle should be sterilized before use by boiling for at least five minutes; during this process the piston should be removed from the barrel to prevent cracking by too rapid expan-

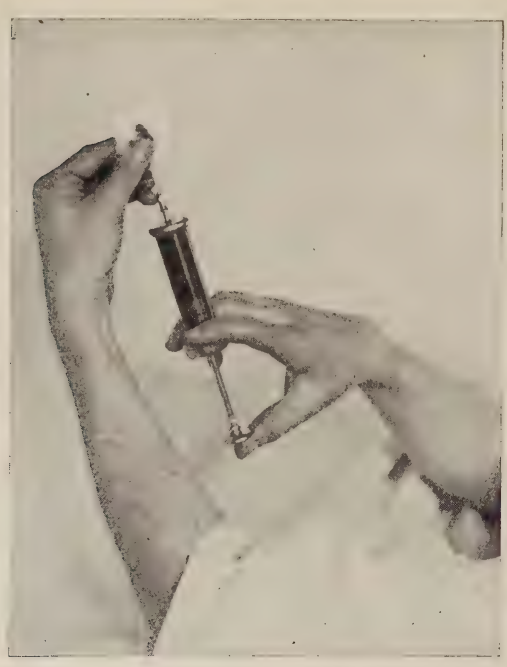

FIG. 50.—AIR BEING EXPELLED FROM A SYRINGE PREPARATORY TO INTRAVENOUS INJECTION. This is done by first attaching the needle and expelling air into a pledget of cotton carrying alcohol to collect any fluid that may be expelled.

FIG. 51.—METHOD OF MAKING INTRAVENOUS INJECTION BY MEANS OF A SYRINGE. The vein is being steadied and the needle introduced; gentle suction is then made upon the piston to determine if the needle has been properly introduced, *as shown by a flow of blood into the syringe.*

sion. It is well not to use sodium carbonate in the water; if it is used, the syringe must be thoroughly washed out with sterile water before use. The apparatus should be allowed to cool off before being loaded with the solution of medicament.

Technic of Injection.—The method of injection is as follows:

1. The syringe should be filled, the needle attached and the air expelled (Fig. 50). *The syringe should never be loaded to capacity, however, as it is necessary to give space for the "flash" of blood after the needle has been entered into the vein.*

2. When all is in readiness, the tourniquet is applied and the skin prepared; local anesthesia is not required.

3. The syringe is grasped as shown in Fig. 51; the vein is steadied and the needle entered at an acute angle to the vein.

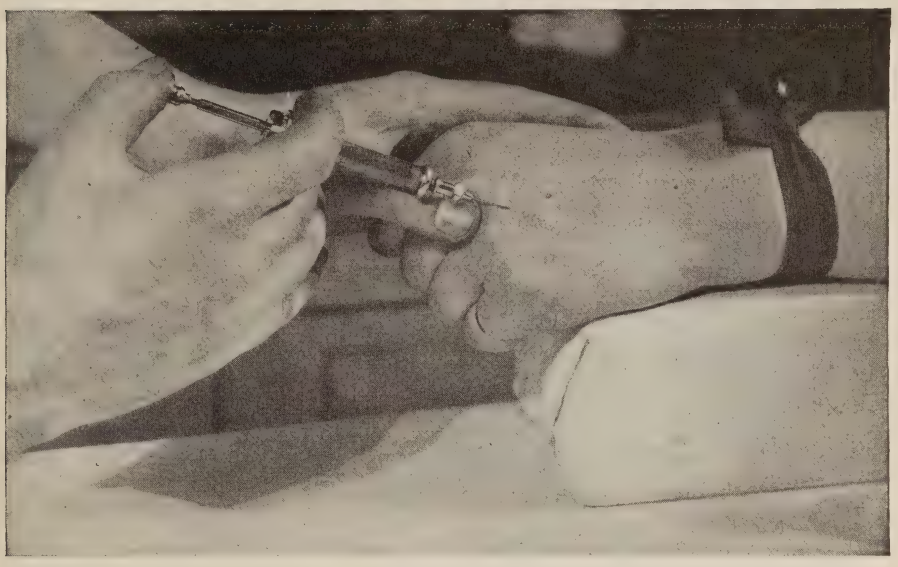

FIG. 52.—INTRAVENOUS INJECTION BY MEANS OF A VEIN ON THE BACK OF THE HAND. A tourniquet is placed about the wrist. A 2 c.c. Record syringe fitted with a No. 22 needle is being employed for injecting 0.6 gm. neoarsphenamin dissolved in 2 c.c. of sterile water.

4. When the needle has entered the vein there is a peculiar "give-away" feeling transmitted to the fingers; the syringe is now deftly grasped by the left hand and gentle suction made on the piston by the right hand.

5. A "flash" of blood is seen in the syringe. The blood should "shoot" in; if it enters slowly the lumen of the needle is not entirely in the vein. Under these circumstances it is necessary to gently rotate the syringe or to advance it a little or withdraw a little, when suction should again be made. Never inject unless the needle is surely in the vein. It is a mistake to inject and expect the patient to decide by the production of pain and swelling. If in doubt release the tourniquet and withdraw the needle. After a minute or two re-apply the tourniquet and try another vein.

6. If the "flash" is satisfactory, release the tourniquet and inject very slowly. If there is a possibility of dislocating the needle by attempts to release the tourniquet it should be left undisturbed as the injection can be

usually slowly given with the tourniquet in place. If pain or swelling are produced stop at once. After some experience has been gained one can tell by the amount of pressure required on the piston whether or not the injection is entirely intravenous.

7. The tourniquet should be released before the needle is withdrawn to prevent perivascular bleeding.

8. During all manipulations care should be taken not to touch or pierce the inside of the vein with the point of the needle, as pain is produced.

9. The position of the patient's hand for infusion by one of the veins on its back, is shown in Fig. 52.

10. The injection of infants by way of the external jugular and temporal veins is readily accomplished (Fig. 53).

FIG. 53.—METHOD FOR SECURING BLOOD AND MAKING AN INTRAVENOUS INJECTION BY MEANS OF A SUPERFICIAL TEMPORAL VEIN.

The patient is an infant 1 month of age. The head is being steadied and a vein compressed by an assistant. The injection of 0.1 gm. neoarsphenamin dissolved in 2 c.c. of sterile water is being given with a 2 c.c. Record syringe fitted with a No. 22 needle (large enough to first obtain a flow of blood before the injection is given).

11. After injection the site of puncture should be compressed and cleansed; as a rule no dressing is required but it is well to have the patient flex and extend the arm vigorously for a few seconds to make sure that bleeding will not occur before the clothing is adjusted.

12. If a 1 or 2 c.c. syringe is used it is well to use a needle of gauge No. 22; with a No. 24 or 28 needle one may not secure the "flash" of blood and dreadfully sore arms and abscesses have resulted from the injection of such highly concentrated solutions into the perivascular tissues.

CHAPTER LX

TECHNIC OF EPIFASCIAL, INTRAMUSCULAR AND SUBCUTANEOUS INJECTIONS

The technic of epifascial, intramuscular and subcutaneous injections appears to be so simple as scarcely worthy of close attention in connection with the subject of the treatment of syphilis. But in view of the fact that the bismuth salts employed in the treatment of this disease are given by intramuscular injection, as well as the growing custom of administering sulpharsphenamin by this and the subcutaneous routes, not to mention the very valuable aid given by intramuscular injections of the soluble and insoluble salts of mercury, the subject has become one of increasing importance, in which an understanding and appreciation of the anatomical considerations and niceties in technic go far toward the prevention of undue pain, tumefactions and dissatisfaction of patients.

Epifascial Versus Intramuscular Injections.—Indeed, while we commonly speak of injections into muscles as "intramuscular" this is not always the case as in some methods an attempt is made to introduce the medicament between large muscles, as between the gluteus maximus and its underlying neighbors, the gluteus minimus and external rotators, as advocated about ten years ago by Wechselmann and Eicke[1] for the comparatively painless and uncomplicated administration of arsphenamin and more recently by Stokes,[2] for the administration of mercury. It is proper to designate injections by this route as epifascial or intermuscular in contradistinction to those that are truly *intramuscular*, in which the medicament is deposited in the muscle itself.

Technic of Epifascial Injection.—According to Stokes, this is the method of choice for the injection of mercury, because it results in less pain and disability as well as fewer tumefactions. I have employed it routinely for the past several years for the injection of mercury and more recently for injections of concentrated solutions of sulpharsphenamin and neoarsphenamin; but I inject suspensions of potassium tartrobismuthate directly into the muscles themselves as bismuth compounds are less painful and less likely to result in unabsorbed tumefactions than mercury.

Anatomical Considerations.—By this method of injection an attempt is made to deposit the medicament in the areolar tissue on the upper surface of the fascia lata covering the gluteus maximus muscle. When a large volume of fluid is deposited in this region, as in the epifascial injection of arsphenamin by Wechselmann's technic, it does not form a tense induration of the body of the muscle or spread laterally to the hip or sacral margin, but presents promptly at the lower border of the buttock in the gluteal sulcus with better absorption and more freedom from local irritation than results from intramuscular injections. Due care must be taken to avoid irritation of the sciatic and branches of the superior gluteal nerves.

Apparatus Required.—A 2 c.c. Record or Luer syringe is required for the administration of mercury, bismuth and small or concentrated doses of sulpharsphenamin, neoarsphenamin and arsphenamin For larger volumes a 10 c.c. syringe may be used. It is very important, however, to use the proper

sized needles which should be of gauge 20 or 22 and 1½ inches long for thin or flabby buttocks, 2 inches for the average adult and 2½ inches for obese individuals. It is imperative that the points be rather long and very sharp; turned points are very painful. The needle should be bright and free of rust; also free of corrosion if a steel needle is employed because of the danger of the shaft snapping off in case the puncture is made with too sudden a thrust, meets with an obstruction or the patient struggles.

Preparation of the Apparatus.—The syringe and needles should be sterilized by boiling for at least 5 minutes, as due precautions must be taken against bacterial injection particularly in the injection of sulpharsphenamin and neoarsphenamin, since the resulting muscle necrosis favors bacterial activity; when mercury is being administered, bacteria are doubtless destroyed if introduced owing to the high bactericidal activity of these compounds including those that are insoluble as the salicylate, calomel and

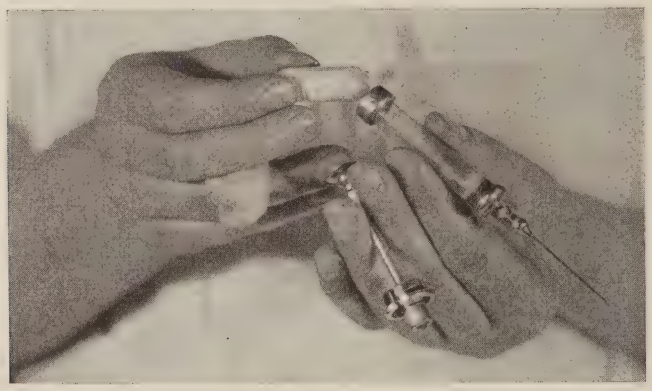

FIG. 54.—LOADING A SYRINGE WITH AN OILY SUSPENSION OF MERCURY OR BISMUTH. The ampule has been well shaken and the neck broken off. The contents are being poured into the barrel of the syringe; a 2 c.c. Record syringe is being employed.

metal itself (gray oil). Potassium tartrobismuthate suspended in oil is very much less bactericidal and due precautions against bacterial infection are required in its administration.

Loading the Syringe.—When suspensions in oil are to be injected, I believe it is a mistake to draw the suspension into the syringe through the needle to be used for making the injection, because when the needle has been entered into the tissues its lumen should be free of any obstruction to the flow of blood in case a vein has been pierced. Any one can satisfy himself that a gauge 20 or 22 needle filled with an oily suspension of the insoluble mercury and bismuth salts ordinarily employed will not readily permit the passage of blood when the syringe is disconnected or even when suction is made on the piston of a syringe loaded with 1 or 2 c.c. of the inoculum. It is a better practice to load the syringe by means of a larger bore sterile needle, as gauge No. 16 or 18, which is then disconnected.

The stock bottle or ampule of suspended drug must be thoroughly shaken before loading in order that one may obtain an even suspension and inject at least approximately the dose decided upon. In this connection I hope that the manufacturers will abandon the practice of putting up ampules with long narrow necks. Not infrequently the drug is deposited in these necks and is displaced only by the most prolonged and violent shaking. A

wide necked ampule can be opened by filling a scratch followed by a smart tap just as easily as a narrow necked ampule.

FIG. 55.—PREPARING AN AMPULE OF SULPHARSPHENAMIN OR NEOARSPHENAMIN FOR EPIFASCIAL OR INTRAMUSCULAR INJECTION.
The extreme tip of the ampule is being filed and broken off.

FIG. 56.—PREPARING A CONCENTRATED SOLUTION OF SULPHARSPHENAMIN OR NEOARSPHENAMIN FOR INTRAVENOUS OR INTRAMUSCULAR INJECTION.
With a syringe and needle about 2 c.c. of sterile water are being *rapidly* injected into an ampule of 0.6 gm. sulpharsphenamin. The ampule is now shaken to dissolve the contents.

When the medicaments as potassium tartrobismuthate, salicylate of mercury, etc., suspended in oil, are dispensed in ampules, I frequently file

off the neck, after thorough shaking and cleansing, hold a cleansed finger over the tip of the syringe (Fig. 54) and empty the oil directly into the syringe aided, if necessary, by a sweeping motion of the arm. In this way one quickly empties the *entire* contents of the ampule into the syringe which is followed

Fig. 57.—Withdrawing a Concentrated Solution of Sulpharsphenamin Prepared in the Ampule into the Syringe Preparatory to Intravenous or Intramuscular Injection.

by the introduction of the plunger, expulsion of air and the attachment of an empty needle ready for injection.

When sulpharsphenamin or neoarsphenamin dissolved in water are to be injected, the tip of the ampule may be filed off as shown in Fig. 55, and

Fig. 58.—Prone Position for Epifascial and Intramuscular Injections.

The arms are hanging over the sides of the table to promote relaxation and the toes are turned in. Showing a point (x) in the upper outer quadrant, about which *epifascial* injections may be made to advantage; the site of choice for *intramuscular* injection is shown by xx.

2 c.c. of sterile water introduced into the ampule with a syringe (Fig. 56). The contents are then dissolved, withdrawn into the syringe (Fig. 57), the air expelled and the injection given. This is a method described by Schamberg and will be referred to again with more detail in Chapter LXII.

Position of the Patient.—Epifascial injections are best given with the patient lying prone on a table and completely relaxed, the arms hanging over the sides and the toes turned in (Fig. 58) as advocated by Stokes. Rigidity and outward rotation of the toes produces dimpling of the buttocks and tends to disturb anatomical relations. Epifascial injections can be given in the standing and even in the sitting positions, but not as easily and certainly as in the prone position, although intramuscular injections are readily given in the standing or sitting positions.

Site of Injection.—The proper site is near the lower inner angle of the upper outer quadrant shown in Fig. 58. In this area one does not run the risk of striking bone or depositing the drug near large nerves with the produc-

FIG. 59.—CORRECT MANNER OF HOLDING SYRINGE FOR EPIFASCIAL AND INTRAMUSCULAR INJECTIONS WITH INDEX FINGER AGAINST THE PISTON TO PREVENT LEAKAGE.

tion of neuralgia or a persistent neuritis. It is likewise less subject to pressure upon sitting and less painful when turning in bed.

Preparation of the Skin.—The skin should be thoroughly cleansed with 1:1000 bichlorid of mercury in alcohol; 5 per cent. alcoholic solutions of iodin or picric acid may be used followed by wiping with alcohol before and after injection.

Technic of Injection.—(1) The left hand is placed flat on the buttock and with moderate pressure is drawn firmly downward toward the patient's heel, thus flattening and fixing the muscle.

(2) The syringe is grasped in the right hand somewhat like a pen with the index finger against the piston to prevent leakage from the needle (Fig. 59).

(3) The needle is introduced to its full length by a quick stroke.

(4) The syringe is now steadied by the left hand while an attempt is made to aspirate by pulling upward on the piston with the right hand (Fig. 60) to determine whether or not a vessel has been punctured by the appearance of blood (of rare occurrence).

FIG. 60.—ASPIRATION TO TEST FOR THE PRESENCE OF BLOOD BEFORE GIVING AN EPIFASCIAL OR INTRAMUSCULAR INJECTION.

FIG. 61.—TECHNIC OF INTRAMUSCULAR INJECTION.

(5) The needle is now held firmly to the syringe and steadied by the left hand while the injection is given with the right hand.

(6) When the syringe has been emptied the needle is *quickly* withdrawn and the site of injection lightly massaged with a pledget of cotton. It is unnecessary to apply a dressing.

Technic of Intramuscular Injection.—The technic of intramuscular injection is quite similar to that just described except that an attempt is made to lodge the inoculum in the midst of the muscle.

Due care must be exercised against depositing the drug in the subcutaneous tissues or in the immediate neighborhood of large nerves and particularly the sciatic and superior gluteal nerves when injections are given in the buttocks. When a series of injections are given I usually permit the patient himself to select the sites of injection and not infrequently they are able to point out exactly the area which produces least pain and disability. Injections should be given alternately in both sides of the buttocks; as a general rule injections on the left side are less painful and usually preferred.

1. The apparatus required is exactly as described above and likewise the preparation by boiling for five minutes. The needle may be gauge 20 to 22, preferably the latter, but the shaft need not be more than 1 to 1½ inches long for the average adult because the injection is to be deposited in the gluteus maximus muscle rather than beneath it, as in the epifascial injection. Due care must be exercised against the use of dull needles with turned points; also against rusty and pitted needles with partially obstructed lumens. A bright, clean, sharp needle is required for the most satisfactory results. The Rosen needle[3] is very useful for the intramuscular injection of children.

2. The syringe should be loaded in the same manner as described above; it is always important to guard against the accidental injection of a vein when administering suspensions and I believe it is well to load the syringe with a large needle (gauge 16 to 18) and replace it with the smaller empty one (gauge 20) for the injection, so that no obstruction will be offered to a flow of blood in case a vein is punctured; or the syringe may be loaded by my method shown in Fig. 54 without a needle.

3. Intramuscular injections can be made, of course, into any large muscle as the deltoid, quadriceps extensor, etc., but the gluteus maximus is the site of choice and employed in the great majority of cases because giving the least pain and disability.

4. The *site of injection* in the buttocks may be the same as described above for epifascial injection, but the majority of my patients select an area a little lower down as indicated by xx in Fig. 58. I have tried higher sites between the crest of the ileum and a horizontal line passing through the apex of the crease of the buttocks, but in my experience these injections are more painful than those given just above or just below this line. Due care should be exercised, however, against giving the injections low down near the crease because of the proximity of the sciatic nerve.

5. The patient may take a relaxed prone position on a table as described above, but the injections are given just as well in the standing position shown in Fig. 61. Injections in the sitting position are less satisfactory.

6. The skin should be wiped with bichlorid alcohol or with iodine followed by alcohol.

7. The syringe is grasped somewhat like a pen with the index finger against the piston to prevent leakage from the needle; it is my custom to slightly stretch the skin with the left hand and quickly thrust the needle into the muscle in a perpendicular position. One may readily estimate the proper depth for depositing the inoculum; care must be taken not to inject subcutaneously. The piston is now drawn backward to make suction for at least ten seconds to draw blood in case a vein has been pierced, but this is of rare occurrence. If there are no evidences of bleeding the injection is slowly given and the needle *quickly* withdrawn after the syringe has been *completely emptied.*

8. The site of injection should be briefly massaged with a pledget of cotton but a dressing is not ordinarily required.

Technic of Subcutaneous Injection.—At the present time sulpharsphenamin is frequently administered by subcutaneous injection in the treatment of syphilis; neoarsphenamin is likewise sometimes administered by this route. Arsphenamin and the soluble as well as the insoluble salts of mercury and bismuth are given intermuscularly or intramuscularly.

The technic is similar to that described above. As a general rule, it is well not to inject too concentrated solutions; for the adult patient the dose of sulpharsphenamin or neoarsphenamin should be diluted in at least 5 c.c. of sterile water, although some physicians have used 1 and 2 c.c.

A Record or Luer syringe fitted with a No. 22 needle may be employed, previously sterilized by boiling for at least five minutes.

Different *sites* may be chosen. As a rule the upper arm is unsatisfactory because of the disability resulting from the tumefaction which always follows subcutaneous injections. The skin of the abdomen below the belt line is a good location; also the skin of the chest below the inner joint of the clavicle. Injections are also given in the back below the lower angle of the scapula.

The skin should be carefully cleansed and the needle entered freely into the subcutaneous tissues. Too superficial injections are very painful. Some physicians first inject a few drops of a sterile solution of novacain, but I incorporate the anesthetic with the solution of medicament as described in Chapter LXII.

After the injection has been given the area should be well massaged; a dressing is not required.

Treatment of Complications from Epifascial, Intramuscular and Subcutaneous Injections.—For excessive pain, swelling and tenderness hot or cold applications of saturated solutions of magnesium sulphate may be used. Occasionally the pain from the injection of the arsenicals may be so severe as to require the administration of morphin. Some individuals develop unusually severe reactions from injections which the majority bear very well. The cause for this peculiar hypersusceptibility to inflammatory changes is unknown, but I suspect that it is due to some disturbance of the ferment-antiferment balance, since the injection of most anything of a bland nature is likely to elicit an excessive local reaction. A series of injections to individuals of this kind is out of the question. Possibly the hypersusceptibility is due in part to syphilitic infection, which is known to increase the sensitiveness of the skin to injections of various substances, and it may be that the simultaneous administration of the iodids intensifies the subcutaneous and intramuscular reactions, as it is known to intensify the intracutaneous reactions to "luetin," agar, and other substances.

Chronic indurations should be gently pinched and massaged by the patient; sometimes an occasional coat of tincture of iodin is of value if tenderness is present.

Abscesses are of rare occurrence. Not infrequently after injections of sulpharsphenamin and neoarsphenamin the parts appear as if an abscess were forming, but under hot or cold applications these usually subside and undergo absorption. Indeed, I believe that part of the good therapeutic effects following intramuscular and subcutaneous injections are due to the absorption of these products of tissue exudation and necrosis in the form of a non-specific protein type of therapy. Abscesses which open spontaneously or require incision should be treated in the usual manner; they are usually sterile and due precautions are required to prevent secondary bacterial infection.

If blood appears in the syringe when intermuscular and intramuscular injections are being given, the needle must be withdrawn and entered in at a new site. To inject an oily suspension under such circumstances would entail grave consequences from pulmonary and possibly cerebral embolism. I have never observed these accidents, but presume that the treatment must be symptomatic and according to circumstances, since nothing can be done for the removal of the emboli themselves.

With due care in the selection of needles and avoiding the striking of bone, the breaking of needles is a rare accident. Children, however, are risks unless their struggling from fright is restrained. When this accident occurs great presence of mind and calmness of action are required. As a general rule, the break occurs at the hub and the physician should immediately lay down the syringe and caution the patient against the slightest move while an attempt is made to pick the shaft out of the tissues. Sometimes a small incision is required. If these attempts are unsuccessful, the needle should be removed surgically with the aid of X-ray pictures for exact localization, as soon as possible and before it is massaged into the deeper tissues by muscular movements.

REFERENCES

1. Münch. med. Wchn., 1914, 51, 535.
2. Medical Record, 1921, April 30.
3. Jour. Amer. Med. Assoc., 1920, 25, 1385.

CHAPTER LXI

TECHNIC OF SPINAL, CISTERN AND VENTRICULAR PUNCTURE AND INJECTION

As stated in Chapter XLII, spinal puncture for the purpose of securing cerebrospinal fluid for examination is now recognized as a very important and valuable procedure for the diagnosis of symptomatic and asymptomatic infection of the central nervous system and as a guide in the treatment of all cases of syphilis. Even though there are absolutely no clinical evidences of neurosyphilis, every patient should have at least one puncture and one thorough examination of the spinal fluid before treatment is terminated in order to guard against the error of dismissing individuals as "cured" when there is present latent invasion and infection of the organs of the central nervous system. Upon this point there is a general agreement of opinion, although, as reviewed in Chapter LXXVI, there are marked differences of opinion on the question of the treatment of neurosyphilis by intraspinal treatments. Of course, the majority of patients naturally dread spinal puncture, but by proper technic the operation can be made almost painless and practically free of danger. In this chapter I shall consider the technic of spinal puncture and intraspinal injections solely from the standpoint of syphilis, without entering into a discussion of spinal puncture and intraspinal medication in other diseases of the meninges, brain or cord.

Contraindications to Spinal Puncture in Syphilis.—During the acute early stages of untreated cases of syphilis, when the spirochetes are likely to be present in the blood, spinal puncture is probably contraindicated because of the danger, theoretically at least, of favoring the localization of

the parasites in the tissues of the central nervous system. According to some investigators, when spinal puncture is performed on the lower animals with removal of a few cubic centimeters of spinal fluid at a time when bacteria are in the blood, there is the possibility of favoring infection of the meninges and it is believed, on the basis of analogy, that spinal puncture during the spirochetemic stages of syphilis may favor localization of the parasites in the organs of the central nervous system. I do not know whether this is true or not in syphilis of the human being. Insofar as experiments on rabbits with acute syphilis of the testicles and scrotum are concerned, I have never been able to show that infection of the meninges, brain and cord was favored by repeated simple punctures with the removal of small amounts of fluids, even in animals castrated after inoculation to promote dissemination of the parasites. In dogs with pneumococcus bacteremia, I have never been able to localize the organisms in the meninges by spinal puncture alone although meningitis has been favored and produced by the intraspinal injection of normal dog serum. I doubt, therefore, if simple spinal puncture with the removal of 5 c.c. of spinal fluid is actually dangerous or absolutely contraindicated during the early untreated stages of syphilis; furthermore, actual harm from such has not been observed or reported upon by numerous syphilologists who have systematically examined spinal fluid in the early stages of the disease. However, the danger of favoring localization of spirochetes in the meninges by spinal puncture during the acute early stages of syphilis when spirochetes are present in the blood by the production of vascular changes and disturbances of the choroid-meningeal plexus, *may* exist and it is well not to resort to spinal puncture at this time until after the first course of treatment which may be relied upon to rid the blood of the parasites. Furthermore, there are no practical indications for examination of the spinal fluid and intraspinal treatments at this stage of disease and in general terms it may be stated that in early syphilis spinal puncture and examination of the cerebrospinal fluid are not required until after the first six months of treatment and probably not until the end of the first year.

In the chronic stages of syphilis, spinal puncture is seldom contraindicated if care and precautions are taken against undue excitement and too great or too sudden release of spinal fluid pressure. I have observed but one fatality in my own experience that may have borne a relation to spinal puncture. This patient was an adult male with syphilis of more than twenty years' standing who developed a fatal hemorrhage in the left anterior frontal lobe of the cerebrum about eight hours after spinal puncture in bed with the removal of 4.8 c.c. of spinal fluid. The pressure was not increased and the puncture was conducted while the patient was lying on the left side without mishap and with a guage No. 19 needle which permitted the slow escape of fluid. Whether the rupture of the vessel was due to a lowering of spinal fluid pressure cannot be stated; I doubt, however, that this was the case and especially since the patient showed absolutely no signs or symptoms of reaction or distress for more than six hours after the puncture, during which time the pressure was doubtless restored to pre-puncture level. However, in cases of suspected vascular syphilis of the brain due care must be exercised in spinal puncture and since this condition may be very difficult or impossible of clinical diagnosis, I take as an indication of the possibility evidences of general advanced arterial sclerosis and chronic myocarditis with increased blood-pressure, and always insist in such cases upon the puncture being done in bed, with the patient lying upon his side and with a small calibre needle to insure a very slow rather than sudden reduction in pressure, followed by at least twenty four hours of rest in bed.

It is stated that great care is also required when a tumor in the posterior fossa is suspected. The rapid withdrawal of fluid, and especially rapid withdrawal with the patient in an upright position, may create sufficient negative pressure in the brain stem to produce hyperemia, hemorrhage and foraminal hernia, which is the engagement of the brain stem in the foramen magnum. In such cases the puncture should always be conducted with the patient lying down, with a small calibre needle to permit a very slow reduction of spinal fluid pressure and not more than 2 or 3 c.c. should be removed, followed possibly by the immediate injection of an equal amount of sterile saline solution to restore the pressure.

Dangers of Spinal Puncture in Syphilis.—Thousands of spinal punctures in cases of syphilis in all stages without mishaps have shown that this operation is safe when properly conducted. It is true that headache not infrequently follows, and likewise nausea and even vomiting in exceptional cases, due to excitement, hyperemia of the meninges, and reflex disturbances of the choroid plexus or to temporary leakage of spinal fluid into the loose epidural tissues, but these are not serious and can usually be avoided altogether by attention to certain precautions and technical details.

As stated above, spinal puncture during the acute early stages of untreated syphilis may favor localization of spirochetes in the central nervous system, but this has not been proved. After an adequate course of treatment, consisting of at least four to six intravenous injections of arsphenamin or neoarsphenamin, this danger is very probably removed and there is no evidence at all that spinal puncture, with the removal of a few cubic centimeters of fluid for laboratory examination, causes an exacerbation of neurosyphilis, although this may occur when intraspinal treatments are given.

Slight pain may be produced if a nerve filament is struck or a dull ache follow for a few hours if bone is punctured, but these cannot be listed as dangers and are of infrequent occurrence. I have never observed bacterial infection of the skin or meninges due to spinal puncture, although such have been recorded and Spiller and Payne[1] have recently described a case of idiopathic epilepsy in whom severe symptoms of meningitis with many polymorphonuclear leukocytes in the fluid appeared within a few hours and spontaneously disappeared after about twenty-four hours; this condition may have been due to infection, although very probably not from the skin, since the usual aseptic precautions were taken.

Spontaneous meningeal hemorrhage after spinal puncture has been reported and studied recently by Goldflam,[2] Meylahn[3] and Herman[4] in which the symptoms are said to have been those of a rapidly developing meningitis due to the diapedesis of blood or puncture of a vein and from which recovery may occur spontaneously in mild cases; but I have personally never seen such in many hundreds of punctures, although the appearance of a few drops of blood during the puncture as a result of piercing a vein in the passage of the needle is not of infrequent occurrence.

It cannot be stated that spinal puncture and the withdrawal of spinal fluid in syphilitics for diagnostic purposes is absolutely devoid of danger, and the operation should never be advised or undertaken hastily and haphazardly; but when the operation is properly conducted, serious accidents are quite rare and for the majority of cases of syphilis the operation may be safely and conservatively advised when the examination will be of value in diagnosis, as a guide to treatment and as a criterion of cure.

Precautions in Spinal Puncture.—Spinal puncture is somewhat more difficult when the patient is lying on his side than when in a sitting posture, but the prone position is to be preferred when the spinal fluid pressure is to be

taken and likewise when it is especially advisable or necessary to draw off the fluid very slowly. In the prone position the pressure is lower, the flow is slower, the patient more comfortable and less likely to develop faintness and nausea from fear, nervousness or sudden reduction in spinal fluid pressure. I believe it to be the position of choice for routine work, although the sitting posture is more likely to be successful and is to be preferred from this standpoint for those lacking in experience in the operation in order to minimize the possible injury to the tissues by repeated but ineffectual attempts to secure spinal fluid.

The needle should not be unnecessarily large. In syphilis the consistency of the spinal fluid is practically that of water and gauge No. 19 or even a slightly smaller needle permits the measurement of pressure and the escape of fluid. In acute suppurative meningitis, larger bore needles are required, but in syphilis it is a mistake to inflict unnecessary pain by a large needle, not to mention the greater tear in the dura and arachanoid permitting the post-puncture escape of spinal fluid into the epidural tissues which is very probably the chief cause of spinal puncture headache.

Spinal fluid should be drawn very slowly in cases of choked disc and when a tumor of the brain is suspected.

It is seldom necessary to remove more than 5 c.c. for all routine tests including total cell count, protein tests, Wassermann and colloidal gold tests. Unless the fluid is under increased pressure, it is not well to remove more than this amount except for therapeutic purposes.

Probably the most important precaution of all is rest in bed for at least eighteen hours after puncture. The chief purpose of this is to avoid spinal puncture headache. As will be discussed later with more detail, it is highly probable that this distressing after effect is due to continued leakage of spinal fluid after the needle has been withdrawn with resulting pressure upon the basilar veins and increased intracranial venous pressure and in the prone position the pressure is reduced and leakage less likely to occur than when the patient is up and about. Spinal puncture may be done in the clinic or office but I believe the patient should always be advised to return home as quickly as possible and stay on his back for at least eighteen hours; it is far better to conduct the puncture in the bedroom so that this position may be taken at once.

Technic of Spinal Puncture.—With these preliminary statements, the technic of spinal puncture may be considered and I hope the following description will be of special aid to those physicians lacking experience in this minor operation which has become an important and almost necessary part of the modern diagnosis and treatment of syphilis.

Apparatus Required and Preparation.—The *needle* should be gauge 19 to 20, free of rust, with a short bevel and very sharp (Figs. 62 and 63). Even in the hands of an expert a dull needle with a turned point inflicts unnecessary pain and not infrequently failure to secure spinal fluid. Steel needles may be employed, although some prefer platinum needles because softer and less likely to break in case of violent muscular movements by the patient. My own preference, however, is for the former and I believe it is well to discard a needle or have it sharpened after using it several times. A bright, clean, rust-free, perfectly straight, sharp needle is the first requisite for success in conducting spinal puncture and it is a mistake to use crooked, rusty, dull needles. The bevel should be short rather than long, as otherwise a portion of the fluid may be lost by leakage in the extradural tissues.

Greene[5] has recently advised the use of a gauge 23 needle with a rounded point like a cambric needle to be used with a syringe for suction, because he

has observed in experiments that this kind of needle produces the smallest tear in the dura and consequently the least chances for post-puncture drainage of spinal fluid. I have never used a needle smaller than 19–20, but am confident that this is a question of considerable importance and feel sure that it is a mistake to use the enormously sized needles and "trocars" so commonly seen in hospitals.

The needle should always be sterilized by boiling for at least five minutes and the lumen must be freely open and free of rust, coagulated blood, etc. After use, the needle should be again sterilized as spirochetes are not infre-

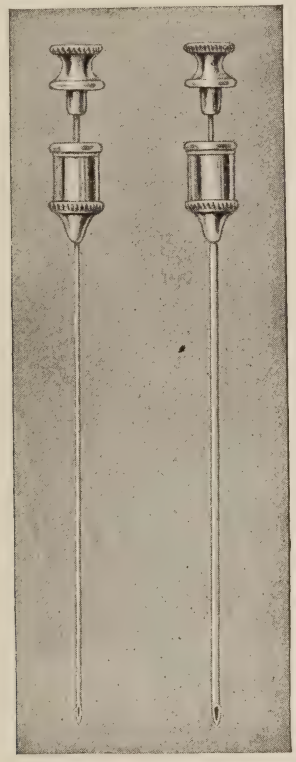

Fig. 62.

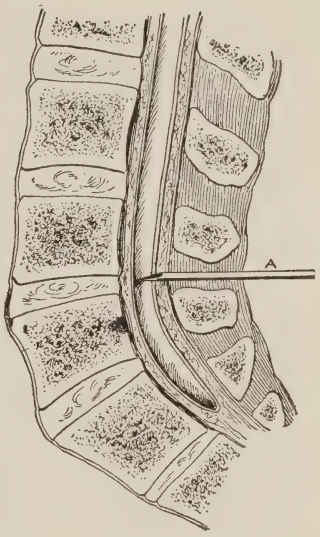

Fig. 63.

Fig. 62.—Babcock Needles for Spinal Puncture.

The needle on the left is gage No. 19 with an inside diameter of 1 mm., and is recommended for routine spinal puncture and intraspinal injections in syphilis, since the fluid is of normal consistency. The needle on the right is gage No. 15 and is recommended for spinal puncture in suppurative meningitis with thickened spinal fluid.

Fig. 63.—An Improperly Constructed Needle with the Opening so Long and Beveled that Half of the Opening May be Within the Dural Sac and Half without, Allowing Escape of Fluid into the Epidural Space. (From Frazier's Surgery of the Spine and Spinal Cord, D. Appleton and Co., New York.)

quently present in the spinal fluids of syphilitics; after drying it is well to vaselin the stylet lightly to prevent rusting.

A manometer for measuring the cerebrospinal fluid pressure is also advisable and the mercury instrument shown in Fig. 72 has been found quite satisfactory.

A sterile 1 c.c. syringe fitted with a No 26 needle is required for the intracutaneous and subcutaneous injection of sterile 1-per cent. solutions of

novacain to butyn for local anesthesia. Two sterile test tubes for the fluid, tincture of iodin, alcohol, flexible collodion and cotton completes the list of required materials.

The physician should carefully prepare the hands to guard against bacterial infection or sterilized rubber gloves may be worn. The latter also afford protection against pricking of the fingers and, since the spinal fluid may be infectious from the standpoint of the presence of spirochetes, due care

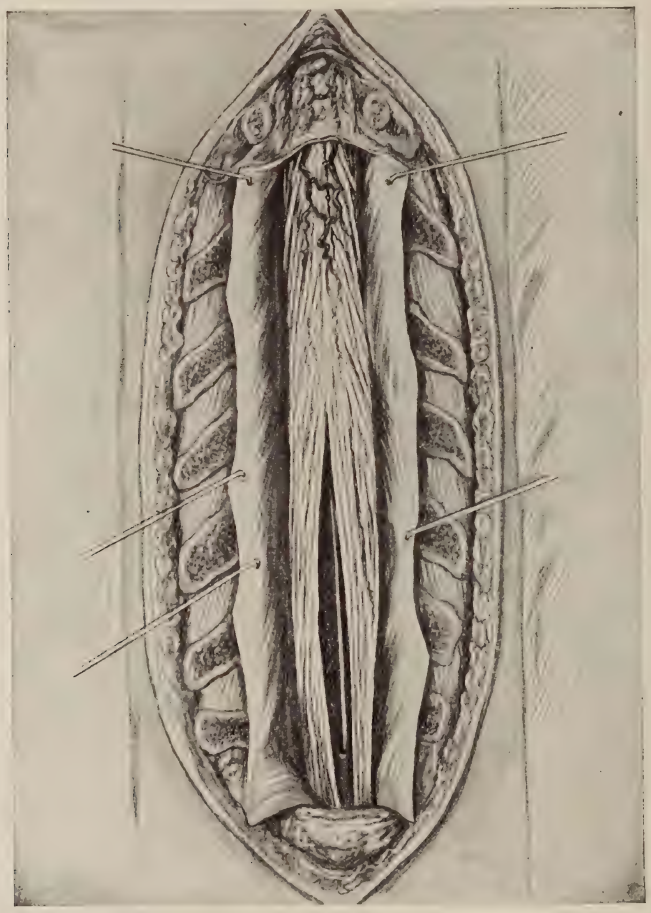

FIG. 64.—ANATOMICAL DRAWING TO REPRESENT THE SPACE BETWEEN THE BILATERAL BUNDLES OF THE CAUDA EQUINA. (From Frazier's Surgery of the Spine and Spinal Cord, D. Appleton and Co., New York.)

is required, although I do not know of any cases of syphilis caused by this means of contamination.

Preparation of the Patient.—In ambulatory patients the most suitable time for puncture is late in the afternoon to permit resting over-night. My practice is to conduct the tests whenever possible during Saturday afternoons which permits most patients to rest in bed over Sunday. It is not necessary to confine the patient for a longer period of time unless headache has followed a previous puncture, in which case a more prolonged stay is advisable.

Special preparation regarding the bowels and diet is not required, although it is well to have had the bowels evacuated and the meal preceding the puncture should be light and taken at least two hours previously. The bladder should be emptied and especially if the patient is nervous.

The skin of the back may be washed with water and soap, followed by alcohol and mercuric chlorid solution, but one or two coats of tincture of iodin followed by swabbing with alcohol just before the puncture is made are sufficient.

Site of Puncture.—Spinal puncture should always be done routinely in the lumbar region between the third and fourth or between the fourth and fifth vertebrae. The tip of the cord (conus medullaris) ends between the first and

FIG. 65.—A HORIZONTAL SECTION AT THE LEVEL OF THE FOURTH LUMBAR VERTEBRA. To illustrate the space between the bilateral bundles of the cauda equina. The lumbar puncture needle should be directed toward this space in the median line to avoid injury to the roots. (Frazier's Surgery of the Spine and Spinal Cord, D. Appleton and Co., New York.)

second lumbar vertebrae but extends a little further down in children, so that puncture between the third and fifth vertebrae will not pierce the cord.

If the patient's back is well arched, there is ample space for passage of the needle between the spinous processes of these vertebrae; it is said that in men these processes are usually directed straight backward and that the needle, therefore, may be directed straight in whereas in women of the same age and build the processes turn downward and that the needle consequently must be directed slightly upward.

Choice of Median or Lateral Routes for Puncture.—Some physicians, probably the majority, enter the needle in exactly the middle line between the vertebrae, while others prefer to enter it from one quarter to as much as one

half of an inch to the right or left of the median line directing the needle upward and inward; Regan[6] has recently reviewed the literature and opinions expressed upon this point and prefers the median route. I always use the median route, not only because it is the easier of the two but for the far more important reason, originally pointed out by Quincke himself many years ago, that the needle is less likely to strike the roots of the cauda equina, since these divide into two laterally placed bundles with a space between them of 2 to 5 mm., as shown in Figs. 64 and 65. This is a point of considerable practical importance because injury of these roots produces not only sharp pain, but has in some instances resulted in more serious motor and sensory disturbances.

When the needle is passed in exactly the middle line, the distance to be traversed before the subarachnoid space and spinal fluid are reached depends upon the age of the patient, the degree of muscular development and panniculus adiposus; in general terms, the distances are as follows, but the matter is of little practical importance, because spinal puncture cannot be performed by passing the needle an arbitrary distance:

Frail adults	4– 6 cm.
Muscular males	6– 8 cm.
Obese adults	10–12 cm.

Position of the Patient.—As previously stated, spinal puncture is easier with the patient sitting than in the prone position; this is especially true of obese individuals. When the pressure is to be taken the prone position on the left side is preferable, because the variations are less and pressures are always expressed under these conditions unless otherwise specified.

An excellent method is to have the patient sitting astride a chair facing its back with a pillow in the pit of the abdomen and the head resting on the folded arms placed on the back of the chair. The position should be perfectly straight without tilting of the pelvis and the back should be arched to give the maximum of space between the vertebral spaces (Figs. 66 and 67). The patient may also sit on the edge of a table or bed with the arms crossed and resting on the knees. If in bed, the patient should lie on either the right or left side with the thighs flexed upon the abdomen, and the shoulders and head forward (Fig. 68) in order to give the maximum arch to the lumbar region; it is very important, however, not to have the shoulders or pelvis tilting or sagging as this very materially alters the direction of the needle (Fig. 69).

Selecting the "Soft" Spot.—As stated above the proper spot for the puncture is exactly in the longitudinal middle line of the body between the third and fourth or between the fourth and fifth lumbar vertebrae. These are called the "soft" spots. By palpating the crests of the ilia and drawing an imaginary line or stretching a sterile towel between these points the "soft" spot between the fourth and fifth vertebrae may be located. As a general rule, the "spot" is just large enough to permit pressure by the index finger; the needle should be entered just a trifle below the middle of the "spot" and exactly in the middle longitudinal line.

Anesthesia.—A general anesthetic is not ordinarily required for adults unless the patient is very nervous and apprehensive when a few drops of chloroform or ether may be given principally for the psychic effect and rarely to the point of anesthesia, because local anesthesia of the skin, as about to be described, renders the operation almost painless. In tabes dorsalis and paresis the operation is peculiarly painless and even local anesthesia may be dispensed with in the case of those patients and particularly men who are not nervous or apprehensive; this always makes the puncture easier and more

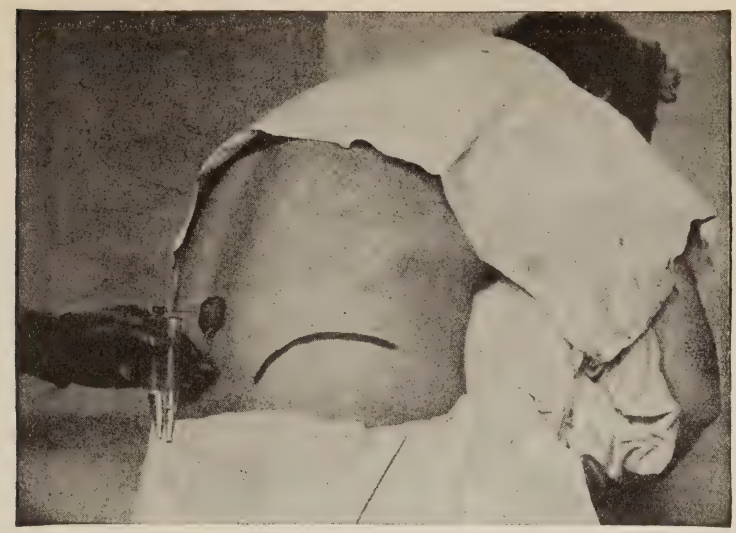

FIG. 66.—SPINAL PUNCTURE IN THE SITTING POSTURE.

The patient is an obese adult male, bent well forward over a folded pillow; the crests of the ilia are outlined. The needle has been passed between the third and fourth lumbar vertebræ in the median line; spinal fluid is being collected in sterile graduated tubes. (Keen's Surgery.)

FIG. 67.—TECHNIC OF SPINAL PUNCTURE IN THE SITTING POSITION AND BY THE MEDIAN ROUTE.

certain with the first passage of the needle, as infiltration of the skin and subcutaneous tissues with an anesthetic solution tends to obliterate the land marks and sometimes deflects the needle.

For local anesthesia a sterile 1-per cent. solution of butyn or novocain may be injected superficially in the prepared skin of the "soft spot," as shown in Fig. 70, until an elevated anemic spot is produced followed by re-entering the needle in a vertical direction in the exact middle of the spot and depositing a

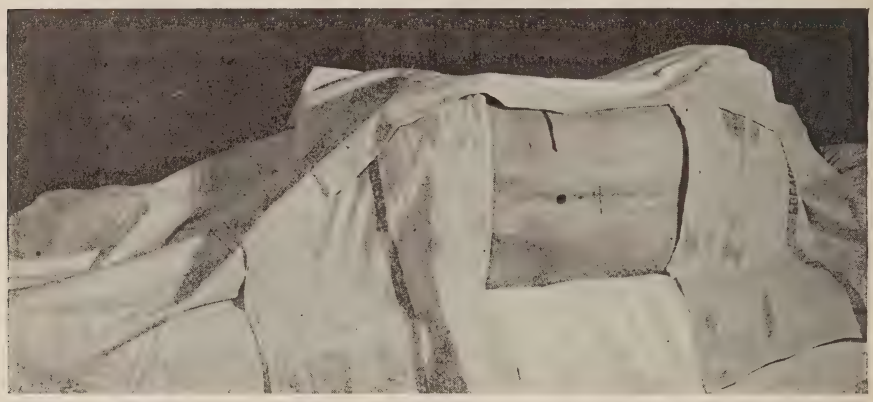

FIG. 68.—PROPER ARRANGEMENT FOR SPINAL PUNCTURE IN THE PRONE POSITION. The back is well arched and perpendicular to the table.

little of the solution as the needle is passed in to its hub. Upon withdrawal, the spinal puncture needle is entered through the same hole in the skin.

Passage of the Needle.—The spinal puncture needle is now firmly grasped about two inches from its tip and passed in exactly the middle line directly inward by gentle pressure (Fig. 71). After the skin has been passed, and with a perfectly sharp needle not larger than gauge 19 this is usually readily

FIG. 69.—IMPROPER ARRANGEMENT FOR SPINAL PUNCTURE IN THE PRONE POSITION. The back is not arched and has slumped forward.

accomplished, whereas with a dull or large needle the skin is apt to wrinkle and deviate the needle from the mid-line, the needle is gently thrust forward slowly and deliberately, rather than quickly and with great force, until it suddenly meets with increased resistance followed by a "give away" sensation which to experienced fingers indicates that the subarachnoid space has been entered. Now, but not before, the stylet may be withdrawn and fluid

FIG. 70.—METHOD OF PRODUCING LOCAL ANESTHESIA IN SPINAL PUNCTURE.

The patient is an obese adult male; the crest of right ilium is outlined and the "soft spot" between the third and fourth lumbar vertebræ located. An *intracutaneous* injection of sterile 1 per cent. butyn is being made with a 1 c.c. Record syringe fitted with a No. 26 needle. The position of the needle will then be changed to the perpendicular and the subcutaneous tissues infiltrated as far as the needle will reach. (Keen's Surgery.)

FIG. 71.—POSITION FOR HOLDING NEEDLE IN SPINAL PUNCTURE.

collected. It is my practice usually to speak to the patient during the passage of the needle in order to conduct the operation in as gentle a manner and with as little pain as possible. I have no patience with or admiration for the almost brutal and abrupt manner with which spinal puncture is frequently conducted on fully conscious, apprehensive but cowed individuals.

If the needle meets with an obstruction it should not be forced as bone has probably been met with and its injury may result in a dull aching pain. The needle should be withdrawn partly or entirely and re-passed.

Every effort should be made to pass the needle successfully with the first attempt and for this reason there should never be undue haste regardless of one's skill in conducting the operation. Every additional passage of the

FIG. 72.—MEASURING THE SPINAL FLUID PRESSURE WITH A MERCURY MANOMETER.

needle required before spinal fluid is secured only adds to the discomfort of the patient and greatly to the likelihood of post-puncture headache.

Measuring the Spinal Fluid Pressure.—The pressure should be measured before there is an escape of more than a few drops of fluid, but one must be sure that the flow is free of obstructions. The method of adjusting the instrument is shown in Fig. 72. As previously mentioned, the patient should be lying on the right or left side, breathing easily and without coughing or other muscular movements. Even a deep breath may slightly increase the pressure and violent excursions of the manometer result from coughing, change of position, etc.

The normal pressure with a mercury manometer is from 4 to 10 mm. with an average 6–8 mm.; a pressure of 12 mm. is border-line while 15 mm. or

higher is distinctly increased. In children the normal pressure is about one-third less. If the pressure is taken in millimeters of water (normal 60–120) it may be expressed in millimeters of mercury by dividing by 13.

A rough gauge of the pressure may be estimated by the rate of flow of fluid from the needle after some experience has been gained with a needle of uniform bore; at least one is able to say whether or not the fluid pressure is distinctly increased or not without being able to detect the finer differences.

The pressure is about one third higher in the sitting posture, the upper limit of normal being about 14 mm. of mercury.

Collection of Fluid.—The fluid is now collected and for this purpose two sterile test tubes should be employed. The first flow is collected in tube No. 1 as it may be blood tinged and unfit for cell counts and protein determinations but acceptable for the Wassermann test; about 2 c.c. should be collected followed by 2 c.c. in tube No. 2 (for cell counts, protein tests and colloidal gold test) and lastly by an additional 1 or 2 c.c. in tube No. 1; this gives a total of from 5 to 6 c.c. of fluid which is ample for all tests. If the fluid runs perfectly free of blood the first few drops may be discarded and about 5 c.c. collected in a single test tube or vial.

The pressure may be taken once or twice during the collection and especially in selected cases in whom a sudden or great drop in pressure is to be avoided. If headache or syncope develop the drainage should be stopped at once.

Dry Tap.—If spinal fluid fails to flow when it is believed that the needle is in the subarachnoid space, it is frequently designated as a "dry tap;" but occurring in cases of syphilis, it may be stated that almost invariably it is due to the fact that the needle is not actually in the subarachnoid space. The needle may be clogged with coagulated blood, a bit of tissue or the opening may be closed by contact with the arachnoid; it should be gently turned, pulled out or advanced very slightly. If these fail it should be withdrawn, inspected and cleaned if necessary and re-passed. In rare cases of old meningitis with adhesions or tumors obstructing the subarachnoid space, "dry tap" or a very scanty flow of fluid may occur, but in the great majority of instances, "dry taps" in syphilis are due to errors or accidents of technic.

After-treatment.—The needle should be rapidly withdrawn, the iodin thoroughly removed with alcohol and the puncture sealed with sterile cotton and collodion.

As previously stated, the patient should remain in bed for at least eighteen to twenty-four hours as originally advised by Quincke.

If the puncture is done in the clinic or office, the patient should be advised to go home immediately, lie down and retire early. As a general rule, it is permissible to get up and resume usual duties next day, but if headache develops upon arising it is usually necessary to lie down again for relief.

Spinal Puncture Headache and Other Complications.—*Soreness* of the back is frequently complained of and especially if several punctures have been made, but this effect is of little consequence and quickly passes away. Sharp *shooting pain* during the puncture is due to striking a nerve root, but is generally relieved by withdrawal of the needle and both pain and numbness quickly disappear. *Nausea, vertigo and syncope* may be due to fright, pain or in exceptional instances, to disturbances of the higher centers by alterations in the spinal fluid pressure. As a general rule, however, these quickly pass off upon the completion of the operation and are treated by the administration of a teaspoonful of aromatic spirits of ammonia in a wineglassful of cold water.

The most annoying of all complications is *headache* and it differs from all others in that, being present when the patient is sitting up, it completely

disappears when he lies down. It is throbbing and severe, and felt mostly in the frontal and occipital regions. No drugs give sufficient relief to allow the patient to walk about and attend to his affairs, or even to sit up for long periods of time, although subcutaneous injections of 1 c.c. of pituitrin have sometimes apparently afforded relief in a few of my patients. The pain comes on quickly when he arises, being fully present in a few minutes; it subsides equally fast when he lies down. In mild cases this situation persists for two or three days; in severer cases it lasts for about seven days with full severity, and then ends abruptly in the course of twenty-four hours.

The *frequency of spinal puncture headache* varies according to conditions. If the punctures are made in the office or clinic and the patients allowed to go home before going to bed, the incidence is from 40 to 50 per cent.; roughly about half of the cases develop headache. If the puncture is followed immediately by resting in bed, the incidence is not higher than 5 per cent. in my experience. Greene, who punctures with a No. 23 gauge needle, and thereby causes the minimum of injury to the membranes, states that his incidence is not higher than 4 per cent.

The *cause of spinal puncture headache* has been a subject of discussion for many years and various theories have been proposed. It has been suggested that it is due to the reduction of pressure by loss of fluid resulting in hyperemia and other changes affecting the choroid plexus, but this cannot be true in all cases, because the incidence does not bear a relation to the amount of fluid drawn and furthermore, headaches have followed in some cases when it was necessary to withdraw the needle before more than a drop or two of fluid was withdrawn. In practicing the method of spinal drainage by Dercum and Gilpin for the treatment of neurosyphilis in which 30 c.c. or more of fluid is withdrawn, I have not observed any higher incidence than in cases from whom 4 to 6 c.c. of fluid was withdrawn for diagnostic purposes, both groups being kept in bed, however, for at least eighteen hours. Nor does it appear that the stage of syphilis materially influences the incidence. It is sometimes stated that during active syphilis the incidence is lower than usual, but MacRobert[7] states that the incidence in his experience in cases of active syphilis was about 37 per cent. as compared with an incidence of 40 per cent. for the whole series.

In all probability the headache is due primarily to a leakage of spinal fluid through the hole in the dura and arachnoid into the epidural tissues, although in a few experiments on dogs I was not able to trace dyes into these tissues after lumbar punctures with gauge 18 needles. MacRobert states that, after lumbar puncture, the hole in the rigid dura is blocked by the delicate arachnoid membrane, but if it clings to the needle and is pulled into the hole forming an invagination or spout, drainage of the spinal fluid continues into the epidural tissues until the hole is healed by tissue proliferation. This results in a loss of normal water-bed support of the brain and when the patient arises the weight of the brain impinges upon the irregular bony surface of the base of the skull which alone may produce pain, but is more likely to be the result of the pressure of the brain forcing the pons on the clivus of the occipital bone, which in turn presses upon the basilar plexus of veins impeding the flow of blood about to leave the skull and forcing it to turn back and travel by other congested channels which results in congestion and a sudden rise of intracranial venous pressure responsible for the headache. When the patient lies down, the weight of the brain is removed from the veins on the clivus and congestion is relieved and in the course of a week the hole in the spinal membranes gradually heals restoring the normal water bed for the brain in the upright position.

In the *treatment of spinal puncture headache*, rest in bed gives almost immediate relief. As previously stated ordinary drugs like antipyrin, caffein, etc., are without appreciable effects. Solomon[8] believes that measures tending to raise cerebrospinal fluid pressure are indicated and observed that the intramuscular injection of 1 c.c. of pituitary extract raised cerebrospinal fluid pressure by 10 to 100 per cent. with permanent and prompt relief in some cases, temporary relief in others and no relief in a few. When the relief is temporary the injection may be repeated as required until the headache disappears spontaneously as it does in all cases. Good effects were also observed from the intravenous injections of 100 to 200 c.c. of sterile distilled water and in the treatment of severe cases it may be well to employ both procedures.

Technic of Cistern Puncture.—This refers to puncture of the cisterna magna and, while it is almost never required for the purpose of securing

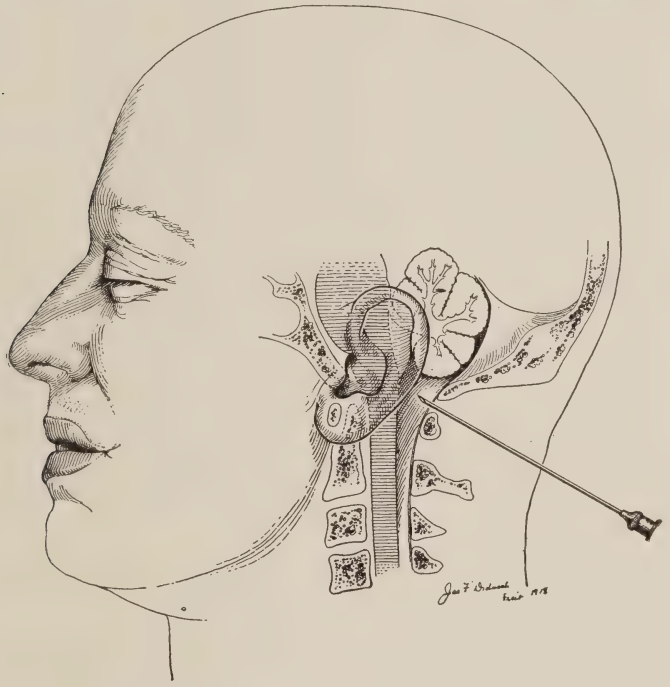

FIG. 73.—TOPOGRAPHICAL STUDY TO SHOW THE LANDMARKS USED IN PERFORMING CISTERNA PUNCTURE.

Composite prepared from numerous tracings of frozen sections. (Wegeforth, Ayer and Essick.)

spinal fluid for laboratory examinations in relation to the diagnosis of syphilis, it is worthy of more attention in relation to intraspinal medication for the treatment of severe neurosyphilis involving principally the cerebral meninges and brain as in paresis; Weigeforth, Ayer and Essick[9] have shown that injections of India ink into the lumbar subarachnoid space of cats were not distributed to these parts but greatly thinned out over the base of the brain and not visible at all over the convolutions, whereas injections into the cisterna magna filled all of the basilar cisterns and extended well over the convexity of the brain.

The anatomical relations of the cisterna magna and general direction of the needle are shown in Fig. 73, as determined by Wegeforth, Ayer and Essick in a thorough investigation of this subject. The puncture is made through the skin, subcutaneous tissues and the occipito-atlantoid ligament which stretches between the posterior rim of the foramen magnum to the corresponding position on the first cervical vertebra.

For introducing the needle a point is selected in the midline of the back of the neck just above the spine of the axis. The glabella and upper edge of the external auditory meatus are valuable for directing the course of the needle, for a line passing through them and the point of insertion on the back of the neck will pass also through the occipito-atlantoid ligament. In thin individuals, palpation over the back of the neck often reveals a deep depression between the occipital protuberance and the spine of the axis, and with the tip of the finger the approximate position of the ligament can be directly located. The distance for the needle to traverse is about 4 cm. rarely exceeding 5 cm. and never less than 3 cm. As in lumbar puncture, however, one must rely upon the tactile sense, the needle giving the same peculiar "give away" sensation when entering the cistern as when it enters the lumbar subarachnoid space.

The same kind of needle may be employed as previously described for lumbar puncture. The preparation of the patient should be the same and in syphilitic cases it is advisable to use local anesthesia as described for lumbar puncture or light ether anesthesia.

The after-treatment is exactly as described for spinal puncture and the patient should be kept in bed for at least eighteen to twenty-four hours.

Technic of Intraventricular Puncture.—Puncture of the lateral ventricles is never resorted to in syphilis merely for obtaining cerebrospinal fluid, but has been advocated as a means for the intraventricular injection of arsphenaminized or neoarsphenaminized serum in the treatment of paresis. According to Hammond and Sharpe,[10] medicaments injected intraspinally do not diffuse over the cerebrum and, even when the injections are made into the cerebral subarachnoid space, they do not diffuse to the opposite hemisphere or down the cord beyond the pons whereas injections of a dye (trypan-blue) into a lateral ventricle were found to diffuse over both hemispheres of the cerebrum, including the ventricles, and throughout the spinal subarachnoid space. From the standpoint of diffusion, therefore, intraventricular puncture is worthy of consideration, although it is questionable whether such injections are worth while in the treatment of paresis, since the spirochetes are not only in the meninges but in the substance of the brain and beyond the reach of spirocheticidal agents in the subarachnoid and ventricular fluids.

Various methods have been described for ventricular puncture, but the procedure is really a major operation, at least for the first puncture, since a small trephine of the skull is required. Under ether anesthesia, Hammond and Sharpe make an incision a little in front of the bregnia and 2.5 cm. from the sagittal sinus and remove a 1.5 cm. button of bone. The dura is incised and a slender cannula with a blunt point is introduced at a point free from cortical vessels and passed downward and a little backward into the lateral ventricle. The head of the table is lowered and from 10 to 20 c.c. of fluid collected. The table is now raised and the injection slowly given by gravity. In second and subsequent injections the scalp is infiltrated with novacain and the puncture made without general anesthesia since the brain is not sensitive.

Technic of Intraspinal Injection.—The injection of arsphenaminized, neoarsphenaminized or mercurialized serum or other substances may be made by attaching a syringe to the needle while in place and giving the injection

very slowly by gentle intermittent pressure upon the piston. For this purpose an adapter may be required for fitting the syringe to the needle being employed; the Record syringe fits the Babcock needles shown in Fig. 62 direct without adapters, and the injection may be made by means of a syringe as shown in Fig. 74.

A much better and safer procedure, however, is injection by gravity since the rate of flow and the pressure are under better control and it is possible to collect spinal fluid for admixture with the inoculum followed by injection of the whole. It is the method of choice for intraspinal, intracistern and intraventricular injections and is widely employed when such therapy is employed.

FIG. 74.—INTRASPINAL INJECTION BY MEANS OF A SYRINGE.

Apparatus Required.—It is my custom to employ a gage 19 Babcock needle for the puncture and for the injection. In addition one requires a 50 c.c. size Record or Luer syringe and about 12 inches of soft rubber tubing fitted with an adapter at one end for the tip of the syringe and a second at the opposite end for the needle (Fig. 75). A 1 c.c. syringe with a No. 26 needle may be required for anesthetizing the skin. All of these instruments are sterilized by boiling for at least five minutes so that the whole operation may be carried out with scrupulous aseptic technic.

Technic of Injection.—After the skin has been prepared and spinal puncture conducted about 5 c.c. of fluid are collected for examination. The barrel of the syringe is then attached to the needle by means of the rubber tubing and from 20 to 40 c.c. of spinal fluid allowed to collect by holding the receptacle low (Fig. 76). The serum is now poured into the barrel, mixed by gentle rotation and agitation and slowly injected (Fig. 77). If the flow is obstructed the piston of the syringe may be used for exerting gentle and cautious pressure in addition to holding the barrel as high as possible. The patient is carefully watched and pressure pains relieved by lowering the receptacle for a few seconds followed by raising it and so on until all has been

FIG. 75.—APPARATUS FOR INTRASPINAL INJECTION BY GRAVITY.

FIG. 76.—DRAINING SPINAL FLUID PREPARATORY TO ADDING SERUM FOR INTRASPINAL INJECTION.

injected. If headache or signs of respiratory or cardiac distress and syncope develop the pressure is reduced by lowering the receptacle and after a few minutes it is generally possible to continue with the injection in a slow and careful manner.

The tubing is now detached or pinched, the needle quickly withdrawn and the wound sealed with cotton and collodion.

After-treatment.—The foot of the bed should be raised and pillows removed for one or two hours in order to favor gravitation of the inoculum to the head. The patient should remain in bed for at least twenty-four hours.

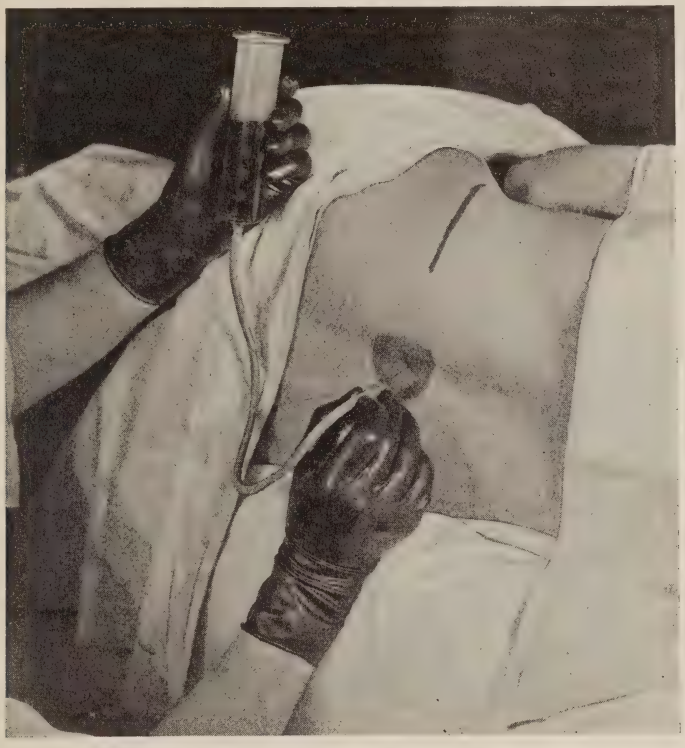

FIG. 77.—INTRASPINAL INJECTION BY GRAVITY.

Pains in the legs are generally due to pressure and tend to disappear within an hour or two. Occasionally they are severe enough to require a small dose of morphin. Headache is likewise due to pressure and may be relieved by an ice-cap.

Technic of Intracistern and Intraventricular Injections.—Exactly the same apparatus and technic may be employed for intracistern injections and, I presume, also for intraventricular injections, although I have had no experience with the latter.

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CHAPTER LXII

TECHNIC OF VARIOUS METHODS FOR THE ADMINISTRATION OF ARSPHENAMIN, NEOARSPHENAMIN AND OTHER ORGANIC ARSENICAL COMPOUNDS

In the administration of arsphenamin and its substitutes, due care should be taken against those errors and faults of administration likely to produce toxic reactions. During the last twelve years various methods have been described with considerable detail so that it may appear almost unnecessary to devote much space to the subject; but there is still considerable confusion in the minds of not a few physicians called upon to administer these compounds regarding various technical points and I shall endeavor in this chapter to describe briefly methods of administration which have yielded uniformly satisfactory results and in which full cognizance is taken of the importance of the methods employed for the preparation and administration of solutions of the compounds to reduce to a minimum toxic or untoward reactions. As stated in 1922 in a bulletin issued by the Hygienic Laboratory,[1] "practically all serious reactions which have occurred in the Government services during the last five years have been especially investigated and in nearly every instance it has been shown that reactions were due (1) to some error in the technic of the preparation and administration of the drug or (2) to faulty examination of the patient, especially in relation to previous injections. In no case has it been possible to prove that the reactions have been due primarily to inherent toxicity of the drug itself."

It is well for the physician administering these compounds to become thoroughly informed upon the importance of factors relating to the drug, its administration and the patient in reference to toxic reactions as already covered in preceding chapters. In this chapter I shall endeavor to describe methods for preparing and administering arsphenamin and neoarsphenamin and other medicinal organic arsenical compounds, but the operative technic of intravenous, intramuscular, intraspinal and other injections are described in Chapters LIX, LX and LXI for the aid and benefit of those physicians giving the injections for the first time, or but occasionally, and who thereby may be lacking in experience with these methods.

General Technic. *Distillation of Water.*—All of the organic arsenicals may be given dissolved in sterile distilled water, but when arsphenamin is to be administered, I believe it is well to employ isotonic saline or glucose solutions to reduce toxicity as far as possible; these solutions, however, should be always prepared of freshly distilled water.

A supply of freshly distilled water is, therefore, one of the most troublesome features connected with the administration of these drugs in private practice. Some firms dispense ampules of distilled water for the administration of concentrated solutions of neoarsphenamin and sulpharsphenamin which are very convenient and satisfactory, since such

water will keep for a year or longer in ampules of very *hard* glass, giving off little or no alkali, as reported by Dreyfus[2] and confirmed by the widespread use of the method in this country.

Distilled water may be kept in flasks for several weeks at a time, provided they are securely sealed with gauze covered cotton stoppers against contamination. The phrase "freshly distilled" is, therefore, only of relative significance, since water distilled weeks and even months before may fulfill the requirements, provided it is stored in flasks of hard glass giving off little or no alkali and sealed against contamination.

As a general rule, physicians may secure supplies of sterile distilled water from a hospital or freshly distilled water from a pharmacy; sterilization is easily accomplished by boiling the water in the flasks or standing them in boiling water up to the level of their contents for fifteen minutes or longer. Expensive and elaborate sterilizers are unnecessary.

Most any glass or tin lined distilling apparatus may be employed providing it is kept clean and in good order; my own preference is for the Barnstead distil which is very compact, requires but little space, is easily kept clean, operates with uniform satisfaction and yields large amounts of distilled water free or almost so of organic matter. For those physicians without a supply of gas, a mo expensive type of instrument may be purchased for use with electricity.

Freshly distilled water collected in sterile flasks is ready for immediate use, but if there is any doubt of its sterility it is better to boil and cool the water before preparing solutions of the drugs. Ordinary good tap water is rendered free of all but traces of organic matter by a single distillation; if, however, the water is known to contain large amounts of organic matter, it is well to re-distil it once more or at the time of the first distillation, one may add to each quart of water a few grains of potassium permanganate which aids in the oxidation and removal of the organic matter.

Preparation of Saline Solution.—This should be prepared of freshly distilled water and chemically pure sodium chlorid; compressed tablets of sodium chlorid are sometimes unsatisfactory. Of course, the solution should be sterile and boiling for five to ten minutes is ordinarily sufficient. Experience has shown that this simple solution is just as satisfactory as Locke's or other solutions more closely approximating the inorganic composition of the blood.

An isotonic or physiological saline solution is 0.7 to 0.9 per cent., or 7 to 9 grams (average 8.5 gms.) of pure sodium chlorid per liter. For the administration of arsphenamin in dilute solution (each 0.1 gm. dissolved in 20 to 30 c.c.), a saline of this strength may be employed; when given in concentrated solution as each 0.1 gm. dissolved in 5 c.c. the saline may be one half this strength, or approximately 0.4 per cent., since each 0.1 gm. of arsphenamin in alkaline solution yields about 0.247 gm. of sodium chlorid.

When neoarsphenamin and sulpharsphenamin are given in dilute solution by gravity it is well to employ saline solution; when given in concentrated solution by a syringe (each 0.1 gm. dissolved in 3 c.c. or less), distilled water alone should be employed since enough inorganic salts are contained in the drugs to render these solutions free of hemolytic activity.

Preparation of Glucose Solution.—If solutions of glucose are employed for the intravenous administration of arsphenamin or neoarsphenamin, the usual isotonic 4.5- to 5-per cent. solutions in water are sufficient after sterilization. Higher concentrations, as up to 50 per cent., have been employed by Rubin[3] and others, but 5-per cent. solutions are sufficient for yielding the possible benefits to be derived from the administration of arsphenamin in glucose solution. It is not necessary, however, to employ glucose solutions for the immediate administration of arsphenamin and its substitutes. The influence of glucose is largely for the purpose of preventing oxidation of the compounds, but this factor is ordinarily under good control when distilled water or saline are employed.

Inspection of the Drugs.—Each ampule should be carefully inspected for cracks; if there is doubt, the ampules may be placed in alcohol for five minutes or longer and if the powder becomes moist it should not be used. This pre-

liminary immersion in alcohol also serves to cleanse the ampule and it should be subsequently laid on a sterile towel or wiped dry with sterile gauze.

Arsphenamin, neoarsphenamin or sulpharsphenamin of a dark brownish color, or otherwise varying from the usual, should not be used. The powders should also be freely movable in the ampules.

Preparation of the Patient.—The amount of preparation required or advisable depends to some extent upon the drug and route of administration to be employed. Constipation should be corrected in all cases, although severe catharsis is uncalled for and unnecessary. Arsphenamin should be administered at least three to four hours after the last meal and preferably late in the afternoon following a light lunch. Neoarsphenamin and sulpharsphenamin may be administered intravenously within a hour or two after a light meal and, when the latter is given subcutaneously or intramuscularly, no special precautions are required at all.

It is well for nervous patients dreading the injections to rest for a while before the treatment is given, and the physician should make a special effort to avoid all unnecessary display of instruments and to maintain a kind and sympathetic but firm attitude. If diarrhea is likely to follow the injection, it is well to administer 10 grains of bismuth subgallate an hour or so after the injection. If the ethereal taste is disagreeable, it is well for the patient to chew a piece of gum during and for a few minutes after injection.

Preparation of the Skin.—The skin may be prepared by applying a coat of 5-per cent. tincture of iodin followed by wiping with alcohol or by wiping with alcohol followed by 1:1000 solution of mercurophen or bichlorid of mercury. When it is necessary to expose a vein by incision, greater care should be taken in the preparation of the skin; but the above suffices for routine work when the needle is to be entered directly through the skin into a vein. With due care local infection of the site is rare and I have never observed or heard of general bacterial infections due to intravenous injections, although local infections with abscess formation have been caused by the introduction of staphylococci apparently from the skin of the patient or the fingers of the physician, following intramuscular injections of the organic arsenical and other antisyphilitic drugs.

Method of Preparation and Intravenous Administration of Dilute Solutions of Arsphenamin.—Whenever possible, arsphenamin should be administered intravenously in dilute alkaline solution by gravity injection; experience in large clinics has shown that this practice results in fewer immediate and early reactions than syringe injections of concentrated solutions.

The Solvent.—Sterile freshly distilled water, sterile physiological saline solution (0.85-per cent.) prepared of freshly distilled water or sterile 5-per cent. glucose in freshly distilled water may be employed; my own preference and practice is to employ saline solution routinely.

Dissolving the Drug.—1. For each 0.1 gm. of compound to be dissolved measure out about 10 c.c. of sterile saline solution or freshly distilled water in an Erlenmyer flask.

2. Wipe the ampule with alcohol or other disinfectant solution and open by filing the neck followed by a sharp blow; sprinkle the powder on the surface of the solvent in small amounts, then gently agitate to prevent the formation of gummy masses, until all has been completely dissolved with the minimum of shaking.

3. If the arsphenamin being employed is difficultly soluble in cold solution, the saline or water may be heated but not boiling; the powder is then added in the same manner in small amounts at a time, due care being taken against the entrance of steam into the ampule which tends to gum the contents.

A better plan with difficultly soluble arsphenamin is to pour all of the powder at once into a dry 100 c.c. glass stoppered cylinder and add 1 to 2 c.c. of pure 95-per cent. ethyl alcohol. The powder is now thoroughly moistened by means of sterile glass rod and cold saline or water added. The cylinder is stoppered and with a few shakes a perfect solution is secured. If one is sure to moisten the powder thoroughly with the alcohol (1 c.c. for 0.4 gm. or 2 c.c. for 0.6 gm.), no difficulty is experienced and this is my routine practice, although there is no evidence that hot saline or water increases the toxicity of any brands of arsphenamin being employed at present in the treatment of syphilis.

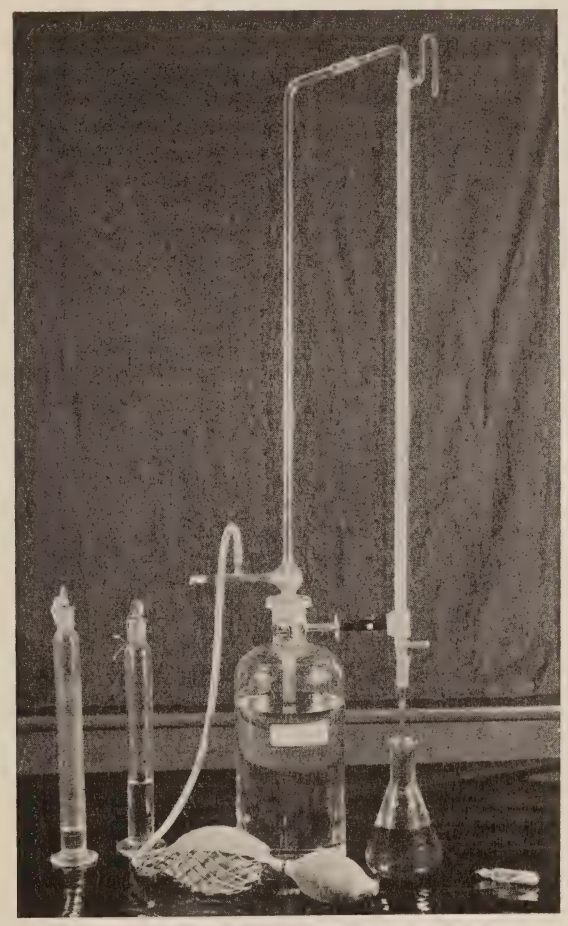

FIG. 78.—AN ALKALINIZING APPARATUS.

Alkalinizing the Solution.—When a perfectly clear solution is obtained it is ready for alkalinization. If for any reason the arsphenamin fails to form a perfect solution, it should be discarded. A clear *normal solution of sodium hydroxid* (approximately 4 per cent.) should be now added all at once in dose of 0.85 to 0.9 c.c. for each 0.1 gm. of drug; this produces a slightly alkaline solution of the disodium salt of arsphenamin which is least toxic.

A 0.4-gm. dose therefore requires 3.6 c.c. of alkali; a 0.6-gm. dose requires 5.4 c.c. The apparatus shown in Fig. 78 is very handy for keeping the

solution of sodium hydroxid free of contact with the air and for measuring it as required. The solution, however, may be kept in a bottle tightly closed with a rubber stopper to prevent the formation of sodium carbonate by the carbon dioxid of the air. The bottle, however, should be of high grade glass and one long used for this purpose; otherwise, one may take a new bottle, place in it a piece of paraffin and heat in a hot air sterilizer until the paraffin is melted. The paraffin should be then evenly distributed throughout the interior and cooled which will prevent the alkali from acting upon the glass.

As the alkali is added the solution becomes very turbid and thick followed by immediate solution with brief shaking. The solution should now be again perfectly clear, and if it is not, a few more drops of alkali may be added. If the solution is not now perfectly clear it should not be used. Excessive shaking should be particularly avoided at this time, because an alkaline solution of arsphenamin is much more readily oxidized than an acid solution.

If too much alkali has been added the solution may be brought back to the neutral point by the careful addition of normal hydrochloric acid; but great care is required and it is better in the interests of safety to discard the solution and prepare another.

Instead of the normal solution of sodium hydroxid, one may employ a 15-per cent. solution as originally advised. It is difficult, however, to know exactly just how much is required since the strength of the solution may be only approximately correct, but small measured amounts may be carefully added until the precipitate has been formed and re-dissolved. When the point of a perfectly clear solution has been reached, one should add one third more of the alkali to the clear solution to produce the disodium salt. As a general rule, the following amounts of 15-per cent. sodium hydroxid are required for this purpose:

0.2 gm. arsphenamin requires 0.5 c.c. or 11 drops of 15 per cent.
0.3 gm. of arsphenamin requires 0.7 c.c. or 16 drops of 15 per cent.
0.4 gm. arsphenamin requires 1.0 c.c. or 20 drops of 15 per cent.
0.5 gm. arsphenamin requires 1.3 c.c. or 25 drops of 15 per cent.
0.6 gm. arsphenamin requires 1.5 c.c. or 32 drops of 15 per cent.

It is to be understood that the amounts of 15-per cent. sodium hydroxid here given are for the purpose of adding approximately one-third excess; therefore, about one third less than the amounts given is sufficient for producing the precipitate followed by clearing and the production thereby of a solution which is mainly one of the monosodium salts of arsphenamin.

Diluting the Solution.—Each 0.1 gm. of arsphenamin should be dissolved at least in 20 c.c. of saline solution or water so that a 0.4 gm. dose requires 80 c.c. and a 0.6 gm. dose 120 c.c. The drug may be dissolved and alkalinized in less saline or water, but should be diluted before administration as the incidence of reactions is diminished by the slow injection of dilute solutions.

Standing to Decrease Toxicity.—The alkalinized solution should now be left standing in a stoppered flask or cylinder for 30 minutes before injection in order to reduce its toxicity. The solution may stand as long as three hours without undergoing any increase in toxicity, providing it is protected from the air, not shaken and the temperature does not exceed 30°C.

Filtration and Administration.—The solution should now be filtered either into a second sterile flask or into the cylinder employed for administration. One may use sterile filter paper, a small wad of sterile cotton or several layers of sterile gauze. This step is always advisable for the purpose of removing minute particles of glass or other foreign material.

The injection apparatus should be sterile and preferably rinsed out with sterile distilled water just before use. The technic of injection is described on page 827. The patient should be lying on a table or bed and the rate of injection should not exceed 25 c.c. per minute so that a 0.4-gm. dose requires a minimum of four minutes for injection and preferably five minutes.

After Care of the Patient.—The site of puncture should be wiped with alcohol or antiseptic solution and sealed with flexible collodion. The patient should rest under observation for at least an hour and preferably several hours eating lightly or not at all for at least four hours and avoiding active movement or excitement.

Method of Preparation and Intravenous Administration of Concentrated Solutions of Arsphenamin.—When the dose to be administered is less than

FIG. 79.—SUITABLE METHOD FOR FILTERING CONCENTRATED SOLUTIONS OF ARSPHENAMIN, NEOARSPHENAMIN AND SULPHARSPHENAMIN.

The vial (on extreme right) measure 90 × 35 cm., is plugged with cotton and sterilized beforehand. The small glass funnel is fitted with a folded filter paper, wrapped in a piece of newspaper and sterilized beforehand by dry heat.

0.4 gm., this method may be employed, although as previously stated the injection of dilute solutions is always to be preferred:

Examine the ampule carefully, cleanse, open and:

1. Empty the contents (0.1 to 0.3 gm.) into a sterile 25 c.c. graduated cylinder.
2. Add 1 c.c. of pure ethyl alcohol and thoroughly moisten all of the powder.
3. Add 20 c.c. of cold or warm sterile water or saline solution, place the stopper and dissolve the drug with the minimum of shaking.
4. *For each* 0.1 *gm. of arsphenamin* add 0.9 c.c. of normal (4 per cent.) solution of sodium hydroxid or 0.25 c.c. of a 15-per cent. solution, using a graduated 1 c.c. pipet.

5. Mix gently; the solution should be perfectly clear. Filter through paper into a sterile vial (Fig. 79).

6. Draw the solution into a sterilized Record or Luer syringe, adjust a No. 22 needle and inject very slowly, using the technic described on page 831. The patient should be preferably lying on a table or bed although injections may be given in the sitting position.

An ampule of 0.4 or 0.6 gm. of arsphenamin may be prepared in this manner with a larger cylinder and by noting in the cylinder the total amount of solution one may inject with the graduate syringe an amount of solution corresponding to the dose which it is desired to administer.

7. The site of puncture usually requires no further attention than briet compression or covering with collodion. The patient should rest under observation for at least one hour and preferably several hours.

Method of Preparation and Intravenous Administration of Dilute Solutions of Neoarsphenamin.—Since most brands of neoarsphenamin now available are of very low toxicity, this method is gradually falling into disuse in favor of the method of concentrated solutions administered intravenously by syringe injection. It cannot be denied, however, that fewer reactions follow the injection of dilute solutions of gravity than syringe injections of concentrated solutions and in large clinics it is frequently possible to give the gravity injections as quickly as by syringe by having one operator handle two tables. the technic is as follows:

1. Examine the ampule carefully, cleanse, open and empty the contents into a sterile graduated cylinder and add at least 10 to 15 c.c. of sterile cold saline solution or water for each 0.1 gm.; or the drug may be emptied into a sterile Erlenmeyer flask and the solution added, although the former method is preferable.

Careful inspection of the drug is even more important than in the case of arsphenamin since the drug decomposes more readily even though there are no cracks in the ampule. The powder should be freely mobile and lemon-yellow to canary-yellow in color. When it approaches a red color, is distinctly lumpy or solidified, it should not be used.

2. Adjust the stopper and dissolve the drug with a minimum of shaking.

3. *Do not use if the solution is not perfectly clear.* Alkalinization is not required.

4. Filter through sterile paper, cotton or gauze to remove foreign matter if present.

5. Inject immediately; it should not be allowed to stand at all, as in the case of solutions of arsphenamin, or never over 20 minutes. For this reason it is well to prepare no more solution than can be administered in this time. The rate of injection should not exceed 25 c.c. per minute; the technic is described on page 827. The patient should be lying on a table or bed and should rest under observation for at least one half to an hour.

Method of Preparation and Intravenous Administration of Concentrated Solutions of Neoarsphenamin and Sulpharsphenamin (First Method).—I have found the following method very satisfactory for the routine intravenous administration of neoarsphenamin and sulpharsphenamin in concentrated solutions and especially for office administration:

1. Carefully inspect the ampule to guard against the mistake of injecting arsphenamin; also for cracks and for changes in the color and mobility of the contents as described above.

2. Cleanse the ampule, open and pour the contents into a sterile graduated glass stoppered 25 c.c. cylinder.

3. Add 20 c.c. of sterile distilled water, stopper and shake briefly to secure a perfect solution.

4. Do not use if the solution is not perfectly clear.

5. Filter through sterile paper into a sterile vial (see Fig. 79).

6. Draw the solution into a sterile 20 or 30 c.c. syringe and inject at once through a No. 22 needle, as described on page 831.

7. The patient may be sitting up with the arm extended. As a general rule, the patient may depart at once and engage in usual activities unless a reaction develops which is of uncommon occurrence with the better brands of both drugs.

Method of Preparation and Intravenous Administration of Concentrated Solutions of Neoarsphenamin and Sulpharsphenamin (Second Method).—The following method devised after that described by Schamberg[4] is likewise very serviceable and may be employed with the sterile distilled water furnished by manufacturers of the compounds:

1. Carefully inspect the ampule and file off the extreme tip to give an opening just large enough for the introduction of a needle.
2. Cleanse and open an ampule of water and draw the contents (usually 5 to 10 c.c.) into a sterile 10 c.c. syringe fitted with a large needle (or the injecting gage No. 22 needle may be used).
3. Expel a few cubic centimeters of water into the ampule of drug rather forcibly; close the opening with a cleansed finger and shake briefly until all has been dissolved.
4. Do not use solutions unless perfectly clear.
5. Draw the solution back into the syringe and mix with the balance of the water.

FIG. 80.—ATTACHMENT FOR FILTERING CONCENTRATED SOLUTIONS OF NEOARSPHENAMIN OR SULPHARSPHENAMIN DURING THE INJECTION.

6. Expel the air and inject intravenously at once. By means of a filter attachment (Fig. 80) the solution may be filtered either while loading the syringe or during injection (former preferred), although this is not absolutely necessary if the solution is perfectly clear.
7. The patient may be sitting with the arm extended as described on page 831. The rate of injection should not be faster than one minute by the watch.
8. As a general rule the patient may go at once and resume usual activities unless a reaction occurs which is rare with the better brands of both compounds.
9. A still more concentrated solution may be obtained by placing 1 or 2 c.c. of sterile distilled water in an ampule as described, dissolving and injecting with a sterile 1 or 2 c.c. syringe fitted with a No. 22 to 26 needle. Even 0.9 gm. of neoarsphenamin or 0.6 gm. of sulpharsphenamin may be administered in this manner and especially when it is necessary to use a vein on the back of the hand, although the more dilute solutions are to be preferred.

Method of Preparation and Intravenous Administration of Silver Arsphenamin.—This compound is really silver sodium arsphenamin which yields a neutral solution and therefore does not require neutralization. *Silver neoarsphenamin* is also employed abroad; both are administered in exactly the same manner as follows:

1. Inspect the ampule, cleanse, open and discharge the contents into a sterile 100 c.c. graduated cylinder.
2. For each 0.1 gm. add 10 c.c. of sterile distilled water or saline solution and dissolve with the minimum of shaking. Do not add alkali.

3. Filter and inject at once. If the dose is not more than 0.2 gm. the injection may be given with a syringe; larger doses should be diluted with saline so that each 0.1 gm. is contained in 20 c.c. and injected by gravity. It is possible, however, to give 0.3 gm. dissolved in 20 to 30 c.c. of saline or water by syringe injection and especially silver neoarsphenamin.

4. The rate of injection should be very slow by either method.

5. The patient should be under observation for at least an hour and should rest quietly for the following few hours.

Method of Preparation and Intravenous Administration of Sodium Arsphenamin.—This compound is not being widely employed at present, but may be administered in either dilute or concentrated solutions in exactly the same manner as neoarsphenamin. Solutions are prepared exactly as described for neoarsphenamin; gravity injection of dilute solutions yields fewer reactions and is to be preferred.

Method of Preparation and Intravenous Administration of Tryparsamid. This substance is usually administered in concentrated solution after the same manner as neoarsphenamin, as follows:

1. Cleanse the ampule, open and discharge the contents in a sterile 25 c.c. graduated cylinder (adult dose usually 1.5 to 3 gm.).

2. Add 10 to 20 c.c. of sterile distilled water or saline and dissolve.

3. Filter and inject intravenously with a syringe fitted with a No. 22 needle as described on page 831. Or the solution may be taken into the syringe and filtered as injected by means of the filter attachment shown in Fig. 80. The rate of injection should be slow, at least one or two minutes being taken.

4. The injection may be given with the patient sitting and usually no immediate toxic reactions are produced.

Sutton Method of Preparation and Intramuscular Administration of Arsphenamin.—Various methods have been advocated for the intramuscular injection of arsphenamin since first introduced by Ehrlich about fourteen years ago, but this route has been largely abandoned within recent years because of the severe local reactions. Sutton, however,[5] has recently described the following technic:

1. Dissolve the arsphenamin in 6 c.c. of sterile water by the aid of rough-surfaced glass beads.

2. Add 4 drops of a 1-per cent. alcoholic solution of phenolphthalein as an indicator.

3. Add a freshly boiled 4 per cent. solution of sodium hydrate, drop by drop with a pipet, meantime gently agitating the mixture, until the resulting emulsion is slightly, but permanently pink in color. Sutton states that it is better not to carry alkalinization too far, as it does not increase therapeutic activity and tends to render the drug more irritating to the tissues.

4. The mixture is then drawn into a sterile syringe, to which is attached a needle at least 2 inches long and injected into the gluteal muscles, care being taken to place it in the middle of the muscle mass. The technic of intramuscular injections is described on page 841.

5. The injections are repeated once every four weeks, four injections forming a series.

6. In making injections by this method I have found that the addition of 2 c.c. of a sterile 2-per cent. solution of butyn to the solution greatly aids in reducing the degree of immediate pain.

Method of Preparation and Intramuscular Administration of Neoarsphenamin.—Neoarsphenamin produces less local irritation than arsphenamin and especially when the solutions are neutral in reaction rather than alkaline. Multiple small doses may be given to infants and children with very satisfactory results and with but slight local reactions. Larger doses in adults invariably produce local reactions of varying severity although the immediate pain may be controlled by incorporating a local anesthetic. The solutions should be concentrated and injected into a large muscle as the gluteus maximum with a sharp needle, rather than in the fascia. The following methods may be employed:

For Infants.—Select an ampule containing 0.1 or 0.2 gm. Inspect carefully, cleanse with alcohol and file off the extreme tip. With a sterile graduated 1- or 2-c.c. syringe fitted with a long, gauge No. 20 or 22 needle, transfer to the ampule 1 c.c. of sterile distilled water for each 0.1 gm. of drug and dissolve. Draw the solution back into the syringe; each 0.1 c.c. contains 0.01 gm. of drug so that a dose of 0.05 gm. requires the injection of 0.5 c.c.; a dose of 0.07 gm. requires 0.7 c.c., etc. Filtration is not necessary. Inject deeply into the gluteal muscles; the Rosen needle is recommended for this purpose, but ordinary gauge No. 20 or 22 needles at least one to two inches in length, depending upon the size of the patient, are satisfactory, due care being taken to prevent excessive struggling.

For Older Children and Adults.—Select an ampule containing 0.6 gm.; inspect carefully, cleanse with alcohol and file off the extreme tip. With a 2-c.c. graduated syringe fitted with a long (1½ to 2 inch) gage No. 20 or 22 needle, very sharp, transfer to the ampule 2 c.c. of a sterile 1 per cent. solution of butyn in water or 2 c.c. of a sterile 2-per cent. solution of stovain in water. Dissolve with the minimum of shaking and draw the solution back into the syringe. Filtration is not necessary. Inject into the gluteal muscles as described on page 841. A dose of 0.9 gm. may be given in the same manner or better by dissolving in 3 c.c. of the anesthetic solution.

The stock anesthetic solution may be kept in a sterile well stoppered Erlenmeyer flask or bottle and it is my practice to add 0.3 c.c. of pure phenol to each 100 c.c. as a guard against contamination.

Very careful aseptic precautions are required to prevent local infection.

Methods of Preparation, Intramuscular and Subcutaneous Administration of Sulpharsphenamin.—Sulpharsphenamin produces less local reaction than either arsphenamin or neoarsphenamin, but is also less therapeutically active in the treatment of syphilis. Intramuscular injections of concentrated solutions are usually less painful than subcutaneous injections. Intramuscular injections are best given in the buttocks, as described on page 841; subcutaneous injections may be given under the skin of the abdomen (above or below the belt line), under the skin of the thorax near the inner end of the clavicle or at the lower angle of the scapula (least desirable because frequently interfering with sleeping on the back). Very careful aseptic precautions are required to prevent infection.

For Infants.—The technic is exactly as described above for the intramuscular injection of neoarsphenamin.

For Older Children and Adults.—The technic is exactly the same as described above for the administration of neoarsphenamin although plain sterile distilled water may be employed instead of the solutions of butyn or stovain. The usual dose is from 0.4 to 0.6 gm.; the former may be dissolved in 1 c.c. and the latter in 2 c.c. giving approximately 30 to 40 per cent. solutions. More dilute solutions may be employed as 0.4 to 0.6 gm. dissolved in 5 c.c. of sterile water, but I believe there is less irritation and discomfort from the more concentrated solutions and especially when administered in 1 to 2 c.c. of sterile 1-per cent. solutions of butyn preserved with 0.3-per cent. phenol, as described above for the intramuscular injections of neoarsphenamin.

Method of Intraperitoneal Administration of Neoarsphenamin.—Since intraperitoneal injections of physiological saline, glucose and sodium bicarbonate solutions and blood have proven useful in the treatment of the diarrheas, acidoses and other diseases of infants, Rosenberg[6] has studied the effects of intraperitoneal injections of neoarsphenamin in rabbits and has given one infant repeated injections with apparent success. I have had no experience with the method, but it is worthy of consideration when intravenous injections cannot be given, although I believe intramuscular injections should yield just as good results with probably less pain. Sanford[64] has

recently reported the successful use of the method in the treatment of ten cases, the usual dose per ten pounds of weight being 5 c.c. of a solution prepared by dissolving 0.15 gm. in 15 c.c. of warm sterile water, every three days for four injections followed by four more at seven day intervals.

Swift-Ellis Method of Intraspinal Administration.—According to Fordyce,[7] the original technic of Swift and Ellis produces least irritation of the lower portion of the spinal cord and is the method of choice; the technic is as follows:

1. Inject intravenously 0.6 gm. of arsphenamin in alkaline solution (disodium salt) as described on page 827.
2. One half hour later remove 40 to 50 c.c. of blood aseptically from a vein of the same or opposite arm; the technic is described in Chapter LIX.
3. Place the blood in a refrigerator over night.
4. Centrifuge and carefully transfer the serum with a sterile pipet to another sterile centrifuge tube and centrifuge again to secure serum free of corpuscles.
5. Pipet the serum to another sterile tube and heat in a water bath for one half hour at a temperature of 56°C.
6. Conduct spinal puncture with the patient on his side and remove about 5 c.c. of fluid for examination. Then attach gravity apparatus and allow 30 to 40 c.c. of spinal fluid to accumulate in the receptacle. Add the heated serum and allow the mixture to slowly re-enter the subarachnoid space by gravity as described on page 858.
7. If neoarsphenamin is injected intravenously instead of arsphenamin, in order to reduce the chances of producing an early toxic reaction, the dose should be 0.9 gm. unless contra-indicated. The intravenous injection may be made by the syringe method. Better results, however, have been observed with intravenous injections of dilute solutions of arsphenamin as described above.
8. The puncture site should be cleansed and sealed with collodion. The patient should remain in bed for at least eighteen to twenty-four hours and longer if a reaction results. For the treatment of untoward effects see Chapter LXI.

Ogilvie Method of Intraspinal Administration.—In this method arsphenamin is not injected intravenously but sterile human serum is arsphenamized *in vitro* according to the following technic, and injected intraspinally:

1. Place 10 c.c. of sterile *clear* human serum in a sterile test-tube in a water bath at 37°C. The serum may be autogenous or that from another individual may be used. It must be sterile, free of corpuscles by one or two centrifugings and likewise free of hemolyzed erythrocytes.
2. In a sterile 100 c.c. glass-stoppered graduated cylinder place 0.1 gm. of arsphenamin. Add 99 c.c. of sterile distilled water at 38°C. and dissolve. Add 0.9 c.c. of *normal* sodium hydroxid and mix gently. The solution is now faintly alkaline and each cubic centimeter contains 0.001 gm. (1 mg.) of arsphenamin.
3. With a sterile 1 c.c. pipet add 0.2 to 0.5 c.c. of arsphenamin solution to the serum and mix. Each 0.1 c.c. of solution carries 0.1 mg. of arsphenamin; the maximum dose is 0.5 mg. of arsphenamin or 0.5 c.c. of the solution.
4. Keep the mixture in a water bath at 37.5°C. for 45 minutes; then place in a thermostat or water-bath at 56°C. for an additional thirty minutes.
5. Inject intraspinally within an hour; under no circumstances inject if more than three hours old from the time it is removed from the last heating.
6. Conduct spinal puncture with the patient on his side. Remove 15 c.c. of spinal fluid (20 c.c. if the pressure is increased). Attach the gravity apparatus (see page 858) and allow 10 c.c. of fluid to accumulate in the receptacle; pour in the arsphenamized serum and slowly inject into the subarachnoid space.
7. The puncture site should be cleansed, sealed with collodion and the patient kept in bed for at least eighteen to twenty-four hours and longer if a reaction (for treatment see Chapter LXI) develops.

Author's Method of Intraspinal Administration.—In 1920 I described a method of intraspinal treatment of selected cases of neurosyphilis[8] which permitted the treatment to be given in a few hours and combining the effects of intravenous medication, as in the Swift-Ellis method, along with the principle of arsphenamizing the serum *in vitro* before intraspinal injection, as in the Ogilvie method.

Unquestionably the Ogilvie method in my experience produces a higher incidence of lower cord irritations than the Swift-Ellis method and the same may be said of my own method, but during the past year and a half I have substituted the use of neoarsphenamin, since the brand I have employed possessed almost the same curative activity as arsphenamin with a much lower toxicity, along with the use of only one-fourth as much sodium citrate, the omission of spinal drainage and the thorough mixing of the neoarsphenamized serum with spinal fluid before injection with the result that the intraspinal injections are as well borne as in the Swift-Ellis method. Furthermore, since using neoarsphenamin general reactions from the intravenous injection are almost unknown, whereas when arsphenamin was injected intravenously it was not unusual for the patient to develop fever, chilliness, etc., which rendered him unfit for the intraspinal treatment. The technic of my modified method is as follows:

1. 0.9 gm. of neoarsphenamin is dissolved in exactly 18 c.c. of sterile distilled water and filtered.

2. One cubic centimeter of this solution is removed to a sterile tube and the balance is injected intravenously with a syringe.

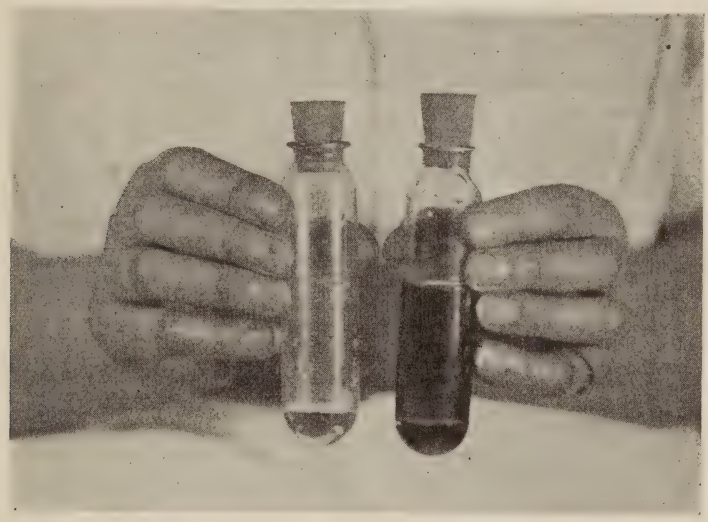

FIG. 81.—SPECIAL CENTRIFUGE TUBE CARRYING SODIUM CITRATE SOLUTION FOR COLLECTION OF BLOOD FOR SECURING SERUM FOR INTRASPINAL INJECTION.

3. A few minutes after injection 25 c.c. of blood is removed with a syringe from the same or opposite arm and placed in a large sterile centrifuge tube carrying 1 c.c. of a sterile 10-per cent. solution of sodium citrate in water (Fig. 81) and gently mixed.

Centrifuge tube on the left carrying 1 c.c. of a sterile 10 per cent. solution of sodium citrate in water; the tube on the right after 25 c.c. of blood has been added.

4. The citrated blood is centrifuged at once and the plasma transferred to a second sterile tube and centrifuged again unless absolutely clear.

5. The cubic centimeter of solution of neoarsphenamin is now diluted with 49 c.c. of sterile saline solution and 1 c.c. added to the plasma. This represents a dose of 0.001 gm. (1 mg.) of neoarsphenamin which is seldom exceeded, although 0.002 gm. has been repeatedly injected without untoward effects.

6. The neoarsphenamized plasma is now heated in a water-bath at 56°C. for thirty minutes.

7. Spinal puncture is done immediately with the patient on his side and about 5 c.c. of spinal fluid collected for routine examinations. With a gravity apparatus about 15 c.c. of spinal fluid is allowed to collect in the receptacle, the neoarsphenamized plasma is poured in and the mixture slowly injected.

8. The puncture site is cleansed, sealed with collodion and the patient is kept in bed for eighteen to twenty-four hours or at least over night.

With this method both intravenous and intraspinal injections are given in two hours or less. It is to be noted that the method as now practiced has been modified from the original by the use of neoarsphenamin, the use of a smaller amount of sodium citrate, the omission of spinal drainage and the thorough dilution of the neoarsphenamized serum with spinal fluid before injection.

Intraventricular and Intracisternal Methods of Administration.—The treatment of neurosyphilis by injection of arsphenamized and neoarsphenamized serum into the lateral ventricles and cisterna magna is still in the experimental stage. Cistern puncture is not much more difficult than spinal puncture, but injections should be made very slowly; in my limited experience I have not observed any better results than those which have followed lumbar injections and I believe the latter are safer. When intracisternal treatments are given, I believe the Swift-Ellis method as described in this chapter is the method of choice because least likely to produce local irritation. If the compounds themselves dissolved in saline solution or serum are injected, I believe that preference should be given sulpharsphenamin and neoarsphenamin because these are less irritating than arsphenamin. The technic of cistern puncture is described on page 857.

Intraventricular injections have been given with success, but the first injection requires a general anesthetic and a small trephine, as described on page 858. I have had no experience with the method but when practiced would advise the Swift-Ellis method as described above for lumbar injections as being least likely to produce irritation although neoarsphenamin has been introduced as in Marinesco's intraspinal treatment without apparent injury.

Method for the Oral Administration of Arsphenamin.—I have already discussed the results of animal experiments and clinical experience by Schamberg and myself[9] with the oral administration of arsphenamin and the indications for this route of administration in selected cases; the formula employed by us for each capsule was as follows:

Arsphenamin	0.03 gm. (gr. ½)
Sodii hydrosulphit	0.015 gm. (gr. ¼)
Bismuthi subgall	0.12 gm. (gr. 2)

A capsule is given after each meal. The sodium hydrosulphite is added to prevent oxidation of the arsphenamin and bismuth is included to correct any tendency to looseness of the bowels. Three capsules a day administers 0.09 gm. arsphenamin and some patients have taken the capsules continuously for a period of six weeks without complaint. Owing to the expense, however, only selected cases can be given arsphenamin by this route although there is no doubt that some therapeutic effects are exerted.

Methods for the Rectal Administration of Arsphenamin, Neoarsphenamin and Sulpharsphenamin.—Within recent years a series of reports have appeared in the literature bearing upon the rectal administration of arsphenamin and neoarsphenamin in the treatment of selected cases of syphilis and especially in nervous or obese women and infants to whom intravenous or intramuscular injections cannot be given. I have already reviewed these reports, but here it may be stated that this route of administration is occasionally useful under certain circumstances as in the case of syphilitics who must travel, individuals who are very obese or with other conditions rendering intravenous injections impracticable; the methods of administration are as follows:

First Method.—Suppositories containing 0.1 gm. of arsphenamin, 0.2 gm. of neoarsphenamin or 0.2 gm. of sulpharsphenamin may be given every other day for six weeks at a

time. Neoarsphenamin and sulpharsphenamin are less irritating than arsphenamin. The inclusion of a very small dose of extract of opium is sometimes required. The suppositories should be inserted after the bowels have been emptied or at least twelve hours before the next evacuation, so that complete absorption may occur. It is my practice to administer them at bed time because the prone position aids in retention.

This method has been particularly advocated for the treatment of congenital syphilis but as recently stated by Littmann and Hutton,[9A] the clinical results obtained by the use of arsphenamin by rectum in children with acute manifestations of syphilis are too feeble to warrant consideration and especially since intramuscular injections of sulpharsphenamin or even of neoarsphenamin are more satisfactory if it is impossible to give these compounds intravenously.

Second Method.—Solutions of arsphenamin, sulpharsphenamin or neoarsphenamin may be injected once or twice a week; neoarsphenamin and sulpharsphenamin are preferred because less irritating. The dose of neoarsphenamin for adults is 1.8 gm., although as much as 4 gm. has been given at one time without irritation; the dose of sulpharsphenamin for adults may be 1.2 gm. The bowels should be evacuated and an enema given one or two hours before the injection. If the bowels are regular, I do not prescribe a cathartic and believe it better not to do so because the injection is sometimes expelled before absorption by subsequent evacuations. But even though the bowels have been evacuated it is well to give the enema an hour or two before the injection of drug. Two ampules of 0.9 gm. each of neoarsphenamin or two ampules of 0.6 gm. each of sulpharsphenamin are dissolved in about 30 c.c. (two ounces) of water. A greased enema tube with a small funnel attached is introduced into the rectum and the solution allowed to flow in gradually as in the administration of an enema, followed by an ounce or two of warm water to wash in all of the drug. Care must be taken against expulsion of the fluid and it is generally necessary for the patient to remain lying down for at least an hour. Patients may be taught to dissolve the drug and to administer it to themselves in their own homes; ordinary tap water may be employed since sterile precautions are not required. As a general rule, the injections should be given at bed time when self-administered by the patient as absorption is practically completed by morning.

Third Method.—Arsphenamin, neoarsphenamin or sulpharsphenamin may be rubbed up into a suspension in one ounce of cotton seed oil and injected into the rectum by the physician followed by an additional ounce of oil to wash in all of the drug. This method is to be preferred when arsphenamin (dose 0.6 to 1.2 gm.) is being administered, as the oil reduces the degree of irritation; with neoarsphenamin and sulpharsphenamin, however, water solutions as described above are usually well borne although if irritation results suspensions in oil are less irritating.

As a general rule, quite a series of injections can be given by any of these methods before the bowel becomes intolerant.

Methods for the Local Application of Arsphenamin and Neoarsphenamin. *For the Treatment of Syphilitic and Frambesial Ulcers.*—As discussed in Chapter XXIII, the local application of arsphenamin and neoarsphenamin in sufficient strength results in the destruction of those spirochetes with which the drugs come in contact. Owing to the expense of the compounds, however, they have not been employed as dusting powders for chancres and other open infectious syphilitic lesions; but I have employed with success dusting powders of one part arsphenamin or neoarsphenamin with two parts of zinc oxid. A powder of one part arsphenamin or neoarsphenamin with two parts of calomel is particularly efficacious for the local treatment of chancres after the secretions have been removed with cotton and warm water. Neither drug produces local irritation when so applied once or twice daily. Alston[10] has reported beneficial results in the treatment of the ulcers of frambesia tropica by local applications of 1:3 powders of arsphenamin or neoarsphenamin. Of course in both syphilis and frambesia, local applications are only of slight value of brief periods of time, since the lesions are usually quickly healed by intravenous injections. But in the treatment of chronic ulcers resistant to treatment local applications are sometimes indicated.

For the Treatment of Spirochetic Gingivitis.—As discussed with more detail on page 362, not a few cases of gingivitis (pyorrhæa alveolaris) show enormous numbers of spirochetes in smears of the secretions. Of course, these are not *Spirochæta pallida*, unless syphilitic mucous patches are present,

but rather an increase of one or more varieties of other species of spirochetes present in the mouth. Apparently these spirochetes are not harmless in cases of this sort and are capable of eliciting severe inflammatory reactions as seen in cases of "trench mouth." Sometimes fusiform bacilli are present indicating a Vincent's infection of the gums.

In all of such cases showing the presence of enormous numbers of spirochetes, the local application of arsphenamin or neoarsphenamin is of distinct value when thoroughly applied at frequent intervals along with the removal of tartar deposits and other surgical procedures.

An excellent method of administration is the local application of 2-per cent. solutions of arsphenamin or 4-per cent. solutions of neoarsphenamin; these are best applied by a dental surgeon who may inject a drop or two of solution deep into each of the pockets by means of a 2 c.c. syringe fitted with a small blunt, curved needle (Fig. 82). The solution should be squirted

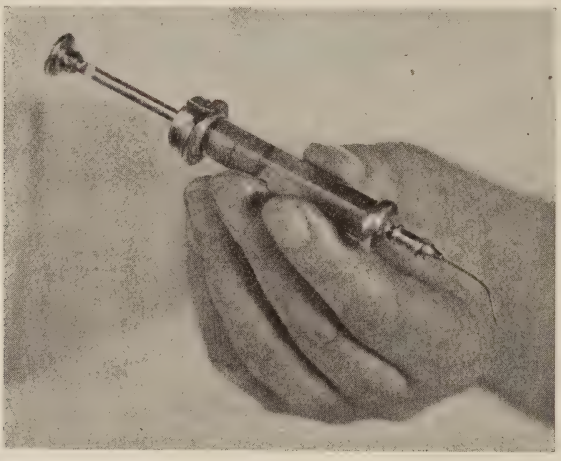

FIG. 82.—SYRINGE AND NEEDLE FOR APPLICATION OF SOLUTIONS OF ARSPHENAMIN IN THE TREATMENT OF GINGIVITIS.

between the teeth and into the pockets in a painstaking manner, due care being observed, however, against injuring the gums with the needle in order to guard against carrying the infection into healthy tissues.

The frequency of applications depends upon the severity of the infection; in acute severe infections daily or twice daily applications are required until improvement results.

The solutions may be prepared as follows:

1. Dissolve 0.6 gm. arsphenamin in 30 c.c. of a hot sterile 50 per cent. solution of glucose. This gives a 2-per cent. solution. It is not necessary to alkalinize the solution as the acid solution is more spirocheticidal and does not oxidize as rapidly. The glucose also aids in preventing oxidation. The solution is now distributed in 1 c.c. glass ampules which may be sealed *in vacuo* by carefully heating the neck until the air is expelled by expansion followed by sealing. Ampules prepared in this manner keep almost indefinitely and even should slight brownish discoloration occur as a result of oxidation, the spirocheticidal properties are not reduced, although solutions which have turned a deep brownish-green-black color should not be employed because so much reduction of the arsphenamin has occurred, that curative properties may be reduced.

2. If neoarsphenamin is employed a 4-per cent. solution may be prepared by dissolving the usual ampule of 0.9 gm. in 22.5 c.c. of cold sterile 50-per cent. solution of glucose in water. The glucose greatly retards oxidation and is preferable to plain water or saline solution. This solution is now distributed in 1 c.c. ampules and sealed *in vacuo* in the same manner.

The local application of these solutions produces no irritation; ordinarily 1 c.c. of either is sufficient for a treatment when carefully applied. The taste may be removed by spraying the mouth with cinnamon water or some similar preparation.

If smears show that many bacteria are likewise present, and most cases of chronic gingivitis are mixed spirochetic and bacterial infections, it is advisable to apply a bactericide at the same time. For this purpose I can highly recommend a solution of mercurophen in conjunction with neoarsphenamin or arsphenamin. All that is necessary is to have on hand a stock solution of 1:500 mercurophen in water which keeps indefinitely; 1 c.c. of 4-per cent. solution of neoarsphenamin or 2-per cent. arsphenamin is drawn into a syringe followed by 1 c.c. of the mercurophen solution. The compounds mix well and may be applied together at the same time. If mercurophen is not available, metaphen or the succinimid of mercury may be employed in similar strength, but other soluble mercurials are precipitated and cannot be used in the same syringe. Not infrequently cases relapse after treatment has been suspended and during the past several years I have been experimenting with dental creams into which arsphenamin has been introduced for prophylactic use by infected individuals. Any good dental cream may be employed, the following formula having proved quite agreeable and efficacious:

Arsphenamin	1.0 gm.
Mercurophen	0.2 gm.
Dental cream	100.0 gm.

This cream contains therefore 1:100 arsphenamin and 1:500 mercurophen; to it may be added 1 gm. of sodium hydrosulphite to reduce oxidation, but the sulphur odor and taste are disagreeable to some individuals and the mixture as given keeps very well in collapsible tubes.

It is poisonous on account of the mercurophen, but not dangerous even though minute amounts are swallowed, although the patient should be warned against swallowing the paste. The mercurophen, however, may be omitted when bactericidal action is not required. Experiments have shown that this cream is highly spirocheticidal and bactericidal when brought in contact with gingival secretions and sputum; it has likewise proven prophylactic for syphilis of rabbits when applied immediately or almost immediately after scrotal inoculations. If the secretions are very acid, the dental cream should be alkaline or made alkaline by the addition of sodium bicarbonate.

I generally advise patients to apply a small amount of the paste with their fingers to the infected portions of the gums, rubbing it in well between the teeth and into the pockets. After a minute or two the teeth and gums are carefully brushed with warm water in the usual manner.

I believe that the routine or frequent use of a preparation of this kind aids in maintaining the gums in a healthy state after the gingivitis has been cured totally or in large degree; it also is useful in the treatment of gingivitis.

For the Treatment of Vincent's Angina.—I have discussed on page 361 with more detail the treatment of Vincent's angina with arsphenamin and its substitutes, but here it may be stated that local applications of these compounds are frequently useful; intravenous injections, however, are sometimes required in very severe cases.

Either arsphenamin or neoarsphenamin powders may be applied directly to the exudates and involved tissues, although solutions are probably better applied and more efficacious. Either 2-per cent. solutions of arsphenamin or 4-per cent. solutions of neoarsphenamin in 50-per cent. glucose solutions may

be employed as described above for the treatment of spirochetic gingivitis. The following solution in glycerin, however, has appeared in my experience to be somewhat better:

Dissolve 0.6 gm. arsphenamin in 15 c.c. of hot 50-per cent. glucose in water; when completely dissolved add 15 c.c. of glycerin and mix. This gives a 2-per cent. solution of arsphenamin in 50-per cent. glycerin and the mixture may be dispensed in an ordinary bottle as it oxidizes but very slowly and may be used until the solution becomes a very dark brown or black color, at which time it should be discarded. If neoarsphenamin is employed, 0.6 gm. should be completely dissolved in 7.5 c.c. of 50-per cent. glucose in water and 7.5 c.c. of glycerin added; this gives a 4-per cent. solution in 50-per cent. glycerin which may be dispensed in the same manner. Plain water may be used instead of glucose solution for either compound, but glucose greatly aids in retarding oxidation.

Either solution should be thoroughly applied with cotton swabs every three hours until the infection is brought under control; the intervals of application may be then lengthened. The disagreeable metallic taste may be removed by rinsing the mouth with diluted *liquor antisepticus* or some similar preparation.

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CHAPTER LXIII

TECHNIC OF VARIOUS METHODS FOR THE ADMINISTRATION OF MERCURY IN THE TREATMENT OF SYPHILIS

Since the toxic and therapeutic effects of mercury vary with the compound selected and route of administration, it is not surprising that a large number of different compounds and mixtures have been advocated for the treatment of syphilis.

Choice of Compounds and Methods of Administration.—With regard to the choice of compounds and methods of administration I can do no better than to quote Fournier: "There is no remedy which suits every case. The choice of mercurial medication should not be based on theoretical considerations, but on clinical indications relative to the peculiarities of the patient and the quality of the disease, which are essentially variable and often impossible to foresee. The best method is that which is tolerated by the patient, and which has an active influence on the symptoms, and this can only be found by experience. Absolutism is out of the question, and the physician

who has the interests of his patient at heart will approach treatment without prejudice and without any preconceived plan, and quite prepared to sacrifice his favorite method for some other which may be more adapted to the indications of the particular case." Again, "if the patient is dyspeptic, or subject to diarrhea, the method of ingestion is contra-indicated, as it may increase the gastro-intestinal intolerance. In certain cases it may be necessary to prescribe several therapeutic agents simultaneously, and under these conditions mercury should be administered externally, leaving the stomach free for other remedies. There are also indications with regard to age. In young children the digestive functions must be respected, which are so vulnerable at this age, and mercurial inunction is better than ingestion at this period of life. Lastly, there are certain private considerations which may contra-indicate certain methods. For instance, if a married man wishes to be treated without the knowledge of his wife, mercurial inunctions will hardly be suitable.

"Therefore, owing to numerous and diverse reasons, the choice of a method of mercurial administration cannot be determined in a general or abstract manner. The choice is subject to the necessities of practice, to special circumstances, and to individual considerations. In fact, everything is subordinate to the indications of the particular case and these indications must always be obeyed."

Method of Ingestion.—The method of ingestion, which is called sometimes the buccal method or the stomachic method, has been unquestionably the favorite method for the administration of mercury; Fournier spoke of it as the "true method of the treatment of syphilis" and Hutchinson likewise had little or no use for other methods.

Advantages and Disadvantages.—This method possesses the distinct advantage of convenience; it is well adapted for the prolonged administration of mercury, that is, with intermittent periods of rest. When prescribed in the form of pills or tablets, the ease of administration removes all fear of detection. As a general rule, there is much less danger of salivation and stomatitis; at least, the administration may be stopped upon the first signs of these with a rapid disappearance of untoward effects. There is likewise less danger of renal irritation and nephritis than in other methods of administration.

On the other hand, gastro-intestinal disturbances are more likely to occur by this route, although it is possible to avoid or correct them in the great majority of cases by a proper choice of compound and regulation of dosage. For this reason the method of ingestion is not well adapted for those patients presenting evidences of gastro-intestinal or hepatic and gall-tract disturbances. Furthermore, for rapid effects the method is inferior to inunction and injection so that we rarely depend upon mercury by any method of administration at the present time in the treatment of acute syphilis, giving preference to arsphenamin or one of its substitutes or even bismuth, and least of all to mercury by ingestion; if mercury must be given under these circumstances, preference is to be given injections or inunctions. Clinical experience and the results of the Wassermann reaction have shown that the therapeutic effects of mercury administered by ingestion are not good or acceptable insofar as the ultimate and complete cure of syphilis is concerned. It is true that the manifest lesions may be dissipated, but the incidence of tertiary lesions, and especially of the central nervous system, are known to be higher than develop in cases of syphilis treated more intensively with mercury or with arsphenamin or one of its substitutes. Mercury by ingestion may be quite worth while along with more intensive treatment with arsenic

or bismuth and at the tail end of prolonged treatment resulting in clinical and serological "cure," as a matter of additional safeguard and security, but the opinion of most syphilologists to-day is that mercury by mouth should not be relied upon for the cure of syphilis to the exclusion of more active forms of mercurial therapy or the administration of more active spirocheticidal agents.

Contra-indications.—The method of ingestion, therefore, is not without certain contra-indications which have been summarized by Fournier as follows:

1. When the patient is affected with some gastro-intestinal disorder or is peculiarly hypersensitive to mercury by this route of administration.
2. When it is imperative not to disturb the digestion of a patient who is in need of nutritional improvement.
3. When it is necessary to leave the gastro-intestinal tract free for other remedies which may be required.
4. When there is pressing and urgent need for rapid or almost immediate mercurialization.

Plan of Administration.—In all cases the administration of mercury should be intermittent, that is, for three to six weeks followed by two to four weeks of rest.

One may give a fixed dose over this period of time, provided it is well tolerated, or one may give ascending doses to determine the individual tolerance followed by the subsequent administration of the maximum tolerated dose for the chosen period of medication. For example, if the protiodid is prescribed in ¼ grain tablets, one may give three a day after meals for two or three days, followed by four a day, five a day, six a day, etc., until signs of enteritis or salivation appear, at which treatment is interrupted for a few days until these symptoms disappear, to be resumed with the maximum dose free of all effects.

Preparations, Doses and Methods of Administration.—A great number of compounds of mercury have been advocated for ingestion, among which the bichlorid, protiodid, mercury with chalk, blue mass and calomel have received most attention. For all practical purposes the first three are sufficient.

The *bichlorid* is said to be less likely to produce salivation and stomatitis; Fournier thought it was especially useful for treatment in the late or chronic stages of syphilis, while the protiodid was to be preferred for the early stages. Bichlorid is more likely than the other preparations to produce gastric disturbances of pain, cramp, and strange sensations—sublimate gastralgia—although intestinal disturbances of cramp and diarrhea are less common than with the protiodid. Women are less tolerant than men—so much so that Fournier thought that sublimate was not a remedy for women. The average dose for men is ½ grain per day (0.03 gm.); for women, about ⅓ grain (0.02 gm.) per day.

The bichlorid may be administered in various ways; one of the oldest formulas for liquid administration is *Van Swieten's liquor:*

℞ Hydrarg. bichlor. corros. ..... 1 gm.
Alcohol ..... 100 c.c.
Aq. distillat ..... 900 c.c.

This is, therefore, a 1:1000 solution, so that a tablespoonful carries about 0.015 gm. or ¼ grain of the compound. The dose is usually a tablespoonful after breakfast and after the evening meal, constituting ½ grain (0.03 gm.) daily. The taste, however, is disagreeable and especially to women, although this may be disguised somewhat by taking the mixture in a wineglassful of milk.

An old favorite is the 1, 2, 3 mixture as follows:

℞ Hydrarg. bichlor. corrosiv. ..... gr. 1 (0.06 gm.)
Potassium iodidi ..... ʒ II (7.2 gm.)
Syr. sarsaparil ..... f℥ III (100 c.c.)

Two teaspoonfuls are given after each meal (¼ grain daily) and possibly again at bedtime (⅓ grain daily).

As a general rule, however, pills are much more convenient and preferable to most patients. A good formula is that of Fournier, a modification of the old Dupuytren pill:

℞ Hydrarg. bichlor. corrosiv.
Ext. opii.....āā 0.3 gm. (5 gr.)
m. Div. Pil. No. XXX

Each pill contains ⅙th grain (0.01 gm.); the dose for men may be a pill after each meal (½ grain or 0.03 gm. daily) and for women a pill after breakfast and the evening meal (⅓ grain or 0.02 gm. daily).

The *protiodid of mercury* (*hydrargyrum iodum flavum* or *viride*) is more likely than bichlorid to produce salivation, but is better tolerated by the gastro-intestinal tract. Sometimes, however, it produces an initial diarrhea, the "diarrhea of apprenticeship" of Fournier, but this is readily checked by temporarily stopping the administration for a few days. Protiodid of mercury is given in pills or tablets, since the compound is insoluble, and is probably the favorite at present for the ingestion treatment of syphilis.

The drug is somewhat better borne than bichlorid and the dose for men may be 1 to 2 grains per day (0.06–0.12 gm.); women require a little less, 1 to 1½ grains per day (0.06–0.09 gm.). Tablets are usually procurable containing ¼ grain (0.02 gm.) each, and 4 to 6 may be given daily in divided doses after meals. It is sometimes advantageous to incorporate opium and especially if there is a tendency to colic and diarrhea; for this purpose Fournier's modification of the old Record pill is to be recommended:

℞ Hydrarg. iod. flav..... gr. xxx (1.5 gm.)
Ext. opii..... gr. V (0.3 gm.)
m. Div. Pil. xxx

Each pill contains about 1 grain (0.05 gm.) of protiodid and ⅙th grain (0.01 gm.) of extract of opium; the dose is 1 or 2 pills a day after meals.

If pills are procured already prepared, it is well to inspect them, because if old and stale they may be very hard and likely to pass through the intestinal canal without absorption.

Mercury with chalk (*hydrargyrum cum creta*) was the old favorite of Sir Jonathan Hutchinson; I have found that it is sometimes better borne than either sublimate or protiodid. Tablets are usually procurable, containing 1 grain in each; Hutchinson usually included 1 grain of Dover's powder as follows:

℞ Hydrarg. cum cret.,
Pulv. ipecac. et opii.....āā grs. xxx (2.0 gm.)
m. Div. Tabl. No. xxx

One pill or tablet is taken after meals; if the mercury is unusually irritating, the dose of Dover's powder may be increased.

If pills are purchased already prepared it is advisable to inspect them, as they may be so hard as to pass through the intestinal canal without absorption; tablets or capsules are to be preferred.

Blue mass (*massa hydrargyri*) and calomel (*hydrargyri chloridum mite*) are not widely employed at the present time for the treatment of syphilis, although either may be administered in dose of ⅙ to ¼ grain three or four times daily. Saxl [1] has recently advocated as 1-per cent. suspension of calomel in dose of twenty drops (approximately ⅙th grain), increased to forty, three times a day after meals. He states that in this dose it is well borne for months at a time and that stomatitis is rare. In my experience, however, colic and diarrhea frequently develop and I believe calomel is quite inferior to the protiodid and mercury with chalk.

Method of Inunction.—This is the oldest method of all for the administration of mercury in the treatment of syphilis and I believe one of the most efficient *when properly applied.* It has been both greatly praised and condemned and undoubtedly possesses distinct disadvantages, but likewise distinct advantages. Many deplorable accidents and fatalities have been ascribed to the method in the old days when patients were subjected to unnecessarily severe treatment with baths, steamings, excessive doses and salivations, but there is no excuse for such gross errors to-day, although it is true that best results are secured when the method is practiced with appropriate bathing and possibly aided by the drinking of sulphur waters.

Advantages and Disadvantages.—The chief advantage of mercurial inunctions are the prompt therapeutic effects, when the method is properly applied and especially by skilled rubbers; the method is particularly desirable for the treatment of severe cases, such as cerebral or spinal syphilis, visceral lesions, and syphilis of the eye threatening vision, when arsphenamin and its substitutes cannot be given. It is a safe method when properly supervised and gastro-intestinal disorders are infrequent. It is painless. It leaves the stomach free for other remedies or for improving nutrition by extra dietary regulations, which is frequently very important in the treatment of syphilis of infants and children.

On the other hand, it is dirty, when the usual mercurial ointment is employed, apt to be repugnant to patients and sometimes compromising from the staining of underclothes, night clothes, bed linens, etc. Furthermore, many patients will not apply it correctly, persistently or consistently, even though they may claim to do so. In other words, it is a difficult method to have done correctly unless minute directions are given or a professional rubber is employed.

It is more likely than other methods to produce salivation and stomatitis, the latter frequently being unusually severe, generalized and of sudden onset, although these ill effects are readily avoidable by due preliminary preparation of the teeth and gums, by daily cleansing of the mouth and by proper regulation of dosage. Inunctions sometimes produce lassitude, vague muscle pains in the back and legs and loss of weight, but such patients are not likely to bear well with mercury in any form of administration.

Inunctions also tend to irritate the skin and may produce various local and generalized forms of dermatitis; the method cannot be applied to those with tender skins, when there is much scarring as after small-pox, or when the skin is thickened, as in ichthyosis.

Preparation, Dosage and Methods of Administration.—As a general rule, the U.S.P. *unguentum hydrargyri* is employed; this is 50-per cent. mercury in suet and lard. The usual adult dose is one dram once a day, but in grave and rebellious cases of syphilis the dose may be increased to two or three drams per day. Infants may receive as much as 15 to 30 grains per day, since they are less likely to develop stomatitis.

The preparation used at Aachen (²) and highly recommended is a 33⅓-per cent. ointment as follows:

℞	Hydrarg. pur	100 gm.
	Adeps lanae	15 gm.
	Ol. olivae	3 c.c.
	Adeps	112 gm.
	Sevum ovis	70 gm.
	Phenolis	3 gm.
	M Div. 60	

This gives 60 doses in waxed papers of 5 gm. each. After a patient has been using these for a time and becomes accustomed to the size of the dose, the ointment may be prescribed in a jar (being cheaper) and the patient trusted to use approximately the correct amount removed with a spoon or the fingers. The phenol may be omitted if objectionable. The adult dose is 5 grams for a twenty minute rub, 7 grams for a thirty minute and 10 grams for a forty minute rub.

At the Hot Springs in this country the blue ointment (50 per cent.) is employed, an ounce being divided into four, six or eight equal parts, one part being used for each inunction. The patient usually takes a hot bath some time after breakfast and then repairs to the "rubbing parlor," where the inunction is applied to the back by a professional rubber for twenty to twenty-five minutes, the patient sitting astride a chair. He then puts on a thin undershirt, which is used as a "mercury shirt" for one week without change, the inunction usually being given daily until signs of salivation are noted.

At Aachen the patient begins in the morning by drinking from one to three glasses of the sulphur water, followed by a bath in a room heated to 70°F. and in water from 95° to

100°F., for ten to twenty minutes, the time varying according to the robustness of general health, being brief for the enfeebled and elderly tabetics. This is followed by rest and breakfast in bed, to be followed soon after by application at the hands of an expert rubber of 5 to 10 grams of the 33⅓-per cent. ointment, the amount varying according to circumstances. As a general rule, the thighs, calves, arms and back are treated in turn from day to day; the hands should never be unpleasantly cold, and it is advisable that all hair be removed from the parts to be rubbed. Unquestionably one of the principal objections to inunctions is the dirty gray color of these preparations and consequently discoloration of the skin and garments. As stated in preceeding chapters, I have found in collaboration with Schamberg, Raiziss and Gavron[3] that inunctions of calomel are apparently absorbed and in practice I have seen salivation and good therapeutic results. I believe, therefore, that calomel ointment may be substituted for blue ointment, although Cole and Littman[4] claim that it is inferior. Since calomel contains 85 per cent. of mercury, the ointment must be stronger in proportion than blue ointment; I have used the following formula for several years:

℞ Hydrarg. chlor. mit..... 130 gm. (℥iv)
Lan.
Adeps benz.....āā 35 gm. (℥ix)
M. Div. 50

The dose is the same as for 50 per cent. blue ointment, being one dram (4 gm.) per day.

Recently Cole, Hutton and Sollmann[5] have advocated a clean method of inunction with the usual blue mercury ointment which consists in rubbing for thirty minutes followed by washing off the skin with benzine and cotton. By this method they found that mercurial inunctions were fully as efficient as when the ointment was kept on the skin. By either this or the old method, about half of their patients required from eleven to nineteen rubs before salivation occurred; about a fourth required a smaller and a fourth a larger number. Unquestionably this is a good method when the matter of objection from the standpoint of the discoloration of the skin, etc., becomes of importance, but, as stated in Chapter LIII, my experiments with rabbits have shown that there is not as much absorption after rubbings with either blue or calomel ointment when the skin is washed with benzine as when the excess is allowed to remain as in the usual method.

In private practice when the inunctions are given by the patient himself or by a member of the family, they are generally most convenient at bed-time and the following routine may be employed:

1. For success the patient must be impressed with the necessity of carrying out the method consistently and persistently. Of the elderly or partially crippled (as in advanced tabes), it is almost useless to depend upon the patient for applying the inunctions; in such circumstances the services of a nurse, professional rubber or a member of the family must be secured. Physicians will do well to keep ever in mind that many patients, even those who are young, robust and with plenty of time to apply the treatment, show a tendency to disregard directions after a few applications unless they are duly impressed with the seriousness of the disease and the danger to future health and well-being of negligence on their part.

2. A hot bath is taken for five to ten minutes, followed by thorough drying.

3. The rubbings are applied to different parts of the body in rotation, to avoid irritation of the skin:

1st day:	left side of thorax.
2nd day:	right side of thorax.
3rd day:	inner surface of the left thigh.
4th day:	inner surface of right thigh.
5th day:	inner surface of left arm.
6th day:	inner surface of right arm.

If applied by a professional rubber or some member of the family, the back may be chosen for the seventh day. The hand may be protected with a rubber glove and nurses and rubbers should be particularly careful to keep their hands and mouths in good condition and to take plenty of out-door exercise.

4. The hand should be warm and the ointment not too stiff or too soft. Hairy portions of the body should be avoided. In male patients it is sometimes advisable to shave the thighs. Inunctions of the thighs and calves of the legs are especially good for tabetics, because of the mental impressions given and the massage of the muscles.

5. The dose is ordinarily one dram of the U.S.P. 50-per cent. mercury ointment, five drams of the 33⅓-per cent. Aachen mercury ointment or one dram of the 85-per cent. calomel ointment. The time of rubbing should be at least 10 to 20 minutes for these doses; larger doses of all three may be used in some cases up to double these amounts, the time of rubbing being increased accordingly up to forty minutes.

6. If mercury ointment is employed, the skin may now be washed off with benzine and cotton and the usual night clothes donned; when this method is practiced, it is well to rub for thirty minutes instead of twenty minutes. I believe, however, that it is better to leave the excess on the skin and to don a thin shirt if the application has been to the thorax or arms, or thin underdrawers or pajamas if the application has been to the legs. The same garment should be worn for at least one week and it is helpful to use lavender colored pajamas to disguise discoloration from mercury ointments. With calomel ointment there is no difficulty at all.

7. Next morning the skin may be bathed as usual and all traces of the ointment removed; it is well to dust the anointed area with talcum powder.

8. The duration of treatment is ordinarily about three or four weeks followed by a rest of two weeks and inunctions again for three weeks until sixty to ninety rubs have been taken, provided they are well borne. It is prudent to discontinue as soon as the gums are affected or the patient debilitated.

9. Inunctions may be taken instead in the morning after a bath, the excess being left on and the same suit of underwear worn for a week, or the excess of mercury may be removed with benzine. It is well, however, to rub for thirty minutes if the latter method is employed.

10. Artificial sulphur water may be taken daily at the same time.

In addition to this method of inunction, *methods of mercurial plasters*, *baths with mercurial soap*, etc., have been advocated and practiced but not usually within recent generations and they are now largely only of historical interest.

Methods of Inhalation and Fumigation.—In the fifteenth century a favorite method of administering mercury was to place the patient in a tent with a brazier to vaporize the drug along with aromatic resins and other substances and allow the half-suffocated victim to inhale the vaporized mercury (method of inhalation) and to have it deposited on the skin (method of fumigation). As practiced at that time along with barbarous preparatory and follow up measures, the method resulted in many fatalities, due principally to absorption of excessive amounts of mercury with poisoning and especially salivation and stomatitis. These methods have, however, come down to the present time but with progressive improvements in technic, so that they are much less dangerous and have some adherents, especially in Europe.

For the inhalation method special apparatus is employed for vaporizing the mercury, calomel or the metal being commonly employed, and breathing it deeply into the lungs. Mercury is deposited in the fauces and a portion is swallowed. I have already discussed in Chapter LII the pros and cons of absorption by this method.

Recently Engelbreth[6] has reported upon the treatment of 1300 cases of syphilis of all stages with more than 60,000 inhalations without one misadventure. A water-bath apparatus was used for volatization in hospital practice and an electric volatizer for home use. In a matter of ten minutes it is claimed that a dose of 0.090 gm. (1⅓ grains) of mercury is inhaled. Various other apparatus have been described from time to time, but I have had no practical experience with any of them and am not in position to express any opinion on their merits or demerits. Doubtless inhalation methods can be made safe and to some extent agreeable, but I surmise great care must be taken of the mouth and throat and against excessive doses since all the accidents of over-dosage ascribed to mercury in general have occurred from inhalations of mercury.

Method of Intramuscular and Epifascial Injection.—Injections of mercury were first practiced by the subcutaneous route, but this method has been almost entirely abandoned in favor of the intramuscular and epifascial routes for both soluble and insoluble salts.

Advantages and Disadvantages.—The epifascial and intramuscular injection of mercury are probably the most popular routes of administration today when prompt and definite mercurialization is required. They insure more

exact dosage than the methods of ingestion and inunction, although when the insoluble salts are injected, absorption is by no means regular or uniform. When the soluble salts are injected, however, absorption is fairly rapid and regular. Injections, therefore, remove the possibility of deceit and deception on the part of patients who may readily enough avoid inunctions or ingestion.

Injections also spare the gastro-intestinal tract and leave it in good condition for purposes of nutrition or the administration of other remedies, although gastro-intestinal disturbances of colic and diarrhea, as well as salivation and stomatitis, may occur when the tolerance of the patient is exceeded.

The chief advantage, however, is the promptness of mercurialization and therapeutic effects and especially when the soluble salts are administered. From these standpoints there can be no denial of the fact that injections are superior to ingestion and inunction.

When the insoluble salts are administered, the injections are given but once a week or ten days and many patients find this practice more convenient and less annoying than other methods of administration.

The disadvantages, however, are the pain, swelling and nodosities with accompanying discomfort and interference with sitting, walking, etc., which may follow intramuscular injections even when administered in a very careful and skilfull manner; this is especially true of those who are lean and spare.

When the soluble salts are administered, an injection is required every day for a month as a course or three times a week for two months, and this may be very irksome to the patient and tends to advertise his malady. Such methods are applicable in hospitals and sanatoriums, but much less so in private practice and especially in small communities where, as Fournier intimates, an individual's business is everyone's business and where a daily visit to the office of a physician is almost sure to be a subject of comment and gossip.

Soluble versus Insoluble Compounds.—The soluble salts are absorbed more promptly and uniformly; therefore, explosive toxic effects, as sudden salivation, stomatitis or nephritis are much less likely to develop than when the insoluble salts are injected. The soluble salts in water or saline, however, are apt to produce more pain and tenderness, although these may be greatly reduced by administering them dissolved or suspended in a vegetable oil like almond oil or palmitin. Their chief advantages, therefore, are rapidity and intensity of therapeutic effects with better control on the prevention of ill effects; their chief disadvantages are the frequency (daily or thrice weekly) with which they must be given.

The insoluble salts mostly employed at present are the salicylate, calomel and gray oil. Calomel undoubtedly gives the best therapeutic results, but likewise the most pain and nodosities; the salicylate is by all odds the most popular at present of the insoluble preparations.

Their chief advantage is the infrequency of injections, being administered not more frequently than once a week or once every ten days unless smaller doses are given biweekly. The old method of giving 3 to 6 grains of calomel at one time at long intervals has been abandoned, because of the fearful explosive reactions of stomatitis, gastro-enteritis and even fatalities that attended this practice. For most patients an injection once a week of the salicylate in dose of 1 grain is a very agreeable and convenient method of taking mercury.

The chief disadvantage aside from the pain and resulting disability is irregular absorption. As shown by Schamberg, Raiziss and myself[7] the mercury contained in them is just as poisonous as in the soluble salts, but it is yielded up to the circulating fluids at a very slow rate. We have shown that in the muscles of rabbits about 50 per cent. of the mercury of calomel, the salicylate and gray oil remains inabsorbed in the body, even as late as 6 or 7 weeks after injection. The daily rate of absorption in these animals was calculated at about one to one and a half per cent. a day, and weekly injections therefore may be progressively cumulative. Doubtless however, absorption is more rapid in human beings as the result of more exercise and muscular movements.

Methods of Administration.—As stated above subcutaneous injections are no longer practiced. The injections may be given intramuscularly or epifas-

cially (intermuscularly) as described in Chapter LX, preference being given the muscles of the buttocks.

Preparations, Doses and Methods of Administration of the Soluble Salts.—Of the soluble salts the bichlorid, succinimid, benzoate, cyanid and mercurosal are most employed although a host are to be found mentioned and advocated in the literature.

Of these the *succinimid* is probably one of the best; the dose is ⅙ to ¼ grain (0.01 to 0.015 gm.) daily, the following formula having been used by me for several years with uniform success:

℞	Hydrarg. succinimid	2.6 gm. (gr. xl)
	Butyn	0.5 gm. (gr. vii)
	Aq. sterilat	100.0 c.c. (f ℥iiiss)

Of this solution 1 c.c. (16 minims) contains approximately 0.02 gm. or ⅓ grain; 0.5 c.c. (8 minims) contains 0.01 gm. or ⅙ grain and is usually the first dose for adults; if well borne, subsequent doses may be of 0.8 c.c. (10 minims) containing 0.015 gm. or ¼ grain daily for 20 to 30 injections. It is not necessary to heat the mixture for sterilization since the succinimid is bactericidal in this concentration. The preparation may be kept in a rubber-capped bottle which is very convenient because graded doses may be removed as required, or it may be dispensed in individual ampules of 1 c.c. each. The *succinimid* may be also dispensed in a vegetable oil and injected once a week; I believe this gives less pain and is frequently more convenient for the patient. The dose, however, may be larger; I have employed the following formula with satisfactory results:

℞	Hydrarg. succinimid	6.0 gm. (gr. 100)
	Butyn	0.5 gm. (gr. vii)
	Ol. amyd. exp	100.0 c.c. (f ℥iiiss)

Sterilization by heat is not required. The mixture may be kept in a rubber capped bottle or dispensed in 1 c.c. ampules. The adult dose is 1 c.c. (16 minims) containing 1 grain (0.06 gm.) per week; the dose for children is 0.2 to 0.5 c.c. once a week. If bi-weekly injections are given the dose should be one half these amounts.

When the *bichlorid* is employed the following formula may be used:

℞	Hydrarg. chloridi corrosiv	0.5 gm. (gr. viii)
	Sodii chloridi	1.0 gm. (gr. xvi)
	Butyn	0.5 gm. (gr. vii)
	Aq. sterilat	100.0 c.c. (f ℥iiiss)

Heating for sterilization is unnecessary because of the powerful germicidal activity of the bichlorid. The dose for an adult is 1 c.c. (16 minims) which contains 0.005 gm. or about $\frac{1}{12}$ th grain, each day for a series of 20 to 30 injections.

A better plan is to administer bichlorid in oil because the absorption is slower and there is less pain: the injections may be given once or twice a week and are therefore frequently more convenient for patients than daily injections. The dose, however, may be larger as follows:

℞	Hydrarg. chloridi corrosiv	3.0 gm. (gr. xiv)
	Butyn	0.5 gm. (gr. vii)
	Ol. amyd. exp	100.0 c.c. (f ℥iiiss)

The ordinary adult dose is 1 c.c. (16 minims) which contains 0.03 gm. or ½ grain once a week; if bi-weekly injections are given the dose is 0.5 c.c. (8 minims) which contains 0.015 gm. or ¼ grain. For infants the dose is 0.2 c.c. (3 minims) to 0.5 c.c. (8 minims) once a week which contains $\frac{1}{10}$th gr. (0.006 gm.) to ¼ grain (0.015 gm.).

The *benzoate* of mercury is also widely employed; it is not readily soluble in water but dissolves upon the addition of sodium chlorid. The following formula is quite satisfactory:

℞	Hydrarg. benzoat	2.0 gm. (gr. xxx)
	Sodii chloridi	2.5 gm. (gr. xl)
	Butyn	0.5 gm. (gr. vii)
	Aq. sterilat	100.0 c.c. (f ℥iiiss)

Sterilization by heat is not required since the solution is bactericidal. The solution may be dispensed in a rubber-capped vial or in 1 c.c. ampules. Each cubic centimeter (16 minims) contains 0.02 gm. or ⅓ grain; the usual daily dose is 0.5 c.c. (8 minims) containing 0.01 gm. or ⅙th grain.

The benzoate may be dispensed in oil for injection once or twice a week; this preparation is also a little less painful:

℞ Hydrarg. benz	6.0 gm.	(gr. 100)
Butyn	0.5 gm.	(gr. vii)
Ol. amyd. exp	100.0 c.c.	(f℥iiiss)

The preparation need not be heated for sterilization as the benzoate is bactericidal. It may be dispensed in a rubber-capped bottle or in 1 c.c. ampules. The adult dose is 1 c.c. (16 minims) containing 0.06 gm. or 1 grain once a week or 0.5 c.c. (½ grain) twice a week.

Preparations, Doses and Methods of Administration of the Insoluble Salts.—Many of the old formulas for the insoluble salts of mercury employed paraffin oil but it is now known that this vehicle is not well absorbed and tends to produce nodosities called "paraffinomas" as recently described by Mook and Wander,[8] Stokes and School,[9] Sutton[10] and others. Vegetable oils are to be preferred such as the expressed oil of almonds, olive oil, cotton seed oil or palmitin; these are saponified in the tissues and more rapidly absorbed with the result that nodosities are now quite uncommon, although they may still develop from the irritation produced by mercury or in the form of traumatic gummas.

Of the insoluble salts of mercury, the *salicylate* is undoubtedly the most popular; I have employed the following formula with success:

℞ Hydrarg. salicyl	6.0 gm.	(gr. 100)
Butyn	0.5 gm.	(gr. vii)
Ol amyd. exp	100.0 c.c.	(f℥iiiss)

Sterilization by heating is not required since the salicylate in this concentration is bactericidal even though insoluble. The mixture may be dispensed in a stock bottle or in ampules but requires thorough shaking to suspend the drug before injection. A heavier base like palmitin may be employed if desired. The dose is 1 c.c. (16 minims) containing 0.06 gm. or 1 grain, once per week or 10 days for six to ten injections. Children are given less in proportion to weight.

Calomel suspensions are also widely employed being more therapeutically active but also more painful; sublimed rather than precipitated calomel is preferable in making up the following formula:

℞ Hydrarg. chlor. mit	5.0 gm.	(gr. 75)
Butyn	0.5 gm.	(gr. vii)
Ol. amyd. exp	100.0 c.c.	(f℥iiiss)

Sterilization by heating is not required since the suspension is highly bactericidal. The mixture may be dispensed in a bottle or in individual ampules, but should be well shaken before injection to secure an even suspension and uniform dose. The usual adult dose is 1 c.c. (16 minims) containing 0.05 gm. or $\frac{9}{10}$th grain once a week or every 10 days for not more than four injections in succession. Children may be injected with smaller amounts proportionate to body weight.

Cole, Littman and Sollmann[10a] have advocated the following formula for either the salicylate or calomel:

Anhydrous lanolin	40 gm.
Distilled water	10 c.c.
Sweet almond oil	150 c.c.
Calomel or mercuric salicylate	29 gm.
Phenol or creosote	20 c.c.
Camphor	40 gm.

The dose of 1 c.c. equals 0.09 or 1½ grains of calomel or mercuric salicylate and is injected intramuscularly once a week.

Metallic mercury in suspension is also widely employed being well known as *gray oil* or *oleum cinereum* (B. P. C.) containing 40 per cent. mercury in very finely divided particles. This strength however, is too high for accurate dosage and requires dilution. If made up from oleum cinereum the mixture may be as follows:

℞ Ol. cinerei	15.0 gm.
Butyn	0.5 gm.
Ol. amyd. exp. q.s	100.0 c.c.

Each cubic centimeter of this mixture (16 minims) contains 0.06 gm. or 1 grain of metallic mercury which is the usual adult dose once a week or once every ten days for a series of six to ten injections. The mixture does not require heating for sterilization but must be well shaken before injection to secure an even suspension and correct dose.

The preparation may be prepared direct from purified mercury according to the following formula, and employed in exactly the same manner:

℞	Hydrarg. pur.	6.0 gm.
	Butyn.	0.5 gm.
	Ol. amyd. exp. q.s.	100.0 c.c.

The dose for an adult is ordinarily 1 c.c. (16 minims) containing 0.06 gm. or 1 grain of metallic mercury, once a week or every ten days for six to ten injections.

Method of Intravenous Injection.—The intravenous injection of mercurial salts was introduced by Bacelli in 1893, but has since acquired but relatively few advocates as a route of administration in the treatment of syphilis, insofar, at least, as the ordinary compounds are concerned. Recently interest in the subject has been renewed since the introduction of flumerin and mercurosal for intravenous medication. Colloidal suspensions of calomel and of metallic mercury have also been advocated and, as stated in Chapter XXX, I have employed mixtures of mercurophen and neoarsphenamin for several years.

Advantages and Disadvantages.—The chief advantage is the rapidity of mercurialization which may be demanded in the treatment of grave cases of syphilis to whom arsphenamin and its substitutes cannot be given on account of intolerance or hypersensitiveness. By throwing the medicament directly into the blood no time is lost in absorption. This, however, may be likewise a disadvantage by reason of rapid elimination in which case a portion of the compound may be eliminated before dissociation or ionization occurs and therefore without exerting therapeutic effects. Additional advantages are mathematically correct dosage and the absence of pain and disability.

However, these injections are not always free of pain and especially if rapidly dissociable compounds like the bichlorid are injected. Phlebitis and thrombosis are not infrequent when this salt is injected although less common in the case of the succinimid and relatively rare with flumerin, mercurosal, mercurophen and colloidal suspensions of calomel and metallic mercury. When bichlorid is accidentally injected perivascularly rather severe reactions may follow; perivascular injections of the last mentioned compounds produce much less reaction.

Intravenous injections may produce some immediate reactions of nausea and vomiting and are more likely to produce polyuria and gastro-intestinal disturbances including salivation and stomatitis, than intramuscular injections. The incidence of these, however, can be reduced by injecting smaller doses and by increasing the intervals between injections. An additional disadvantage for many patients is the frequency of injections; they should be given at least every two to three days for at least six injections, which may be very annoying when several series of injections are required.

In my opinion, therefore, the only real indications for intravenous injections of mercury are two, namely (*a*) to avoid local pain since it is possible to give these without producing phlebitis and thrombosis, and (*b*) to secure more rapid effects than possible from other routes of administration. An additional advantage is the possibility of injecting mixtures of neoarsphenamin or sulpharsphenamin with mercury at the same time with the same syringe; for this purpose, however, only mercurophen or flumerin may be used since the bichlorid, succinimid, mercurosal and other salts are precipitated with more or less rapidity.

Technic of Administration.—Intravenous injections of mercurial compounds are given with a syringe fitted with a No. 22 needle as described on page 831.

Preparations, Doses and Methods of Administration.—The *bichlorid of mercury* has probably been used more frequently than other compounds, but is the least desirable of all from the standpoint of producing phlebitis and thrombosis as well as general ill effects. Bastron[11] has recently advocated doses of $\frac{1}{10}$th grain every three days, gradually increased until as much as $\frac{1}{2}$ grain is given robust adult men. As soon as slight soreness of the gums is produced, the dose is held constant, but the intervals increased to one or two injections a week, a course being at least of six weeks duration. It is convenient to employ a stock 1:1000 solution; 1 c.c. of this contains 0.001 gm. and 6 c.c. contains 0.006 gm. or $\frac{1}{10}$th grain, which is the first dose. Subsequent doses, therefore, are easily increased although I doubt if more than 15 c.c. (0.015 gm. or $\frac{1}{4}$ grain) should ever be exceeded; the injections may be given every three to five days with a close watch for salivation and irritation of the kidneys.

Bastron has also advocated the intravenous injection of bichlorid suspended in serum (mercurialized serum) as being a means for reducing the incidence of phlebitis and thrombosis, but this modification is by no means free of these effects. For these injections human serum is much to be preferred to preparations of horse serum, because the latter tends to produce sensitization and serum sickness. The serum of the patient or the sterile serum of some other human being belonging to the same blood group may be employed and the mixtures kept in sealed ampules ready for administration. For preparing the serum one may add to 1 to 5 c.c. the dose of bichlorid: for a $\frac{1}{10}$th grain dose this may be 0.6 c.c. of a 1:100 solution of bichlorid and for the $\frac{1}{4}$ grain dose, 1.5 c.c. of a 1:100 solution. The mixtures become cloudy, but gradually clear if an excess of serum is employed.

In my experience the *succinimid* is much to be preferred to bichlorid. It is less toxic and much less likely to produce phlebitis. I employ a stock 1:1000 solution and begin with 6 c.c. (0.006 gm. or $\frac{1}{10}$th grain); the injections are given twice a week and gradually increased but never beyond 15 c.c. ($\frac{1}{4}$ grain) at one time.

Flumerin is injected in the form of 2-per cent. solutions in sterile water or saline and these are stable for several days. The dose advised by White and his colleagues has been 0.003 to 0.005 gm. per kilo every two or three days. From my experience with the drug I would advise not more than 0.003 gm. every two days for at least six injections as a course, unless signs of intolerance appear. For an adult of 135 to 150 pounds this dose would be from 10 to 15 c.c. of the 2-per cent. solution or 20 to 30 c.c. of a 1-per cent. solution, the latter being my choice. Marked variations in individual tolerance for flumerin have been noted and since these are not always predictable, patients should be carefully watched for toxic symptoms including routine examinations of the urine.

Mercurosal is usually given in dose of 0.1 gm. freshly dissolved in 5 to 10 c.c. of sterile water or saline solution; my preference is for 20 c.c. of saline solution. The injections are given bi-weekly or every two to three days for at least six injections as a course, unless signs of intolerance appear. The drug is dispensed in the form of a powder in ampules and the solutions are prepared as required just before administration.

The insoluble compounds like colloidal suspensions of *calomel* and of the *metal* are not widely employed in this country. Their possible advantages as compared with intravenous injections of the soluble salts are somewhat slower dissociation, slower elimination, greater freedom from toxic reactions and slightly more prolonged therapeutic effects; pulmonary embolism does not occur when suitable preparations are employed and irritation of the veins is uncommon when the compounds are properly injected. The dose of calomel is ordinarily 0.01 to 0.03 gm. for an adult, corresponding to $\frac{1}{6}$th to $\frac{1}{2}$ grain, every four or five days until ten to twelve injections have been given, unless signs of intolerance and renal irritation develop. Podesta[12] has recently advocated suspensions of calomel in concentrated glucose solution for intravenous injection, but I believe that preference is to be given the soluble compounds. A similar preparation of metallic mercury designated as "lueside" or "mercudol" has been reported upon unfavorably by Cole and his associates.[12a]

Combined Intravenous Injections of Arsenical and Mercurial Compounds.—As stated in Chapter XXX, the addition of a small dose of a compatible mercurial compound to solutions of neoarsphenamin appear to increase trypanocidal and spirocheticidal properties, the mercury acting as a catalyzer or activator of the spirocheticidal activities of the arsenic. The mercury itself produces curative effects in addition to those of the arsenic, if the dose is sufficiently large, as in Linsser's method of the treatment of syphilis.

During the past four years, I have injected mixtures of mercurophen and neoarsphenamin in the treatment of syphilis without ill effects and, I believe, with good therapeutic results. My method is to dissolve 0.9 gm. of neoarsphenamin in 5 or 10 c.c. of water and add 15 c.c. of a 1:1000 stock solution of mercurophen. The mixture, which sometimes becomes slightly opalescent, is filtered and injected at once with a syringe once or twice

a week. The dose of mercurophen is 0.015 gm. or ¼ grain, which is well borne and produces on phlebitis or thrombosis. Sulpharsphenamin in dose of 0.4 to 0.6 gm. may be used in exactly the same manner. Alkaline solutions of arsphenamin (disodium salt) are also compatible and I have many times added 15 c.c. of 1:1000 mercurophen to the solution in the cylinder and injected both intravenously without any ill effects whatsoever from the mercury. The succinimid of mercury and mercurosal cannot be used with either neoarsphenamin or sulpharsphenamin, although mercurosal can be used with arsphenamin (disodium salt) if the mixture is injected at once. Flumerin in dose of 5 to 10 c.c. of 2-per cent. solution may be used with any of the three arsenicals in the same manner; the dose is reduced in order to avoid a double reaction from the two drugs. Bichlorid and succinimid, however, cannot be used because of the rapid production of greenish black solutions due to precipitation of mercury, unless such are desired as in Linsser's method employing the former (see page 353).

Method of Intraspinal Injection.—Various methods have been advocated for the treatment of neurosyphilis by intraspinal injections of mercury since Ravaut[13] in 1913 first injected two drops of 0.1- to 1-per cent. solutions of bichlorid and cyanid of mercury. It was soon found, however, that these injections produced considerable irritation and had to be adandoned. I have found that intraspinal injections of mercurophen, bichlorid and mercurochrome in rabbits in doses as small as 0.001 gm. per kilo frequently produce marked irritation of the meninges with congestion and hemorrhages.

In 1914 Byrnes,[14] however, greatly improved the technic by first mixing bichlorid with serum to form the albuminate before injection. This method may be conducted as follows:

1. Remove 50 c.c. of blood from the patient and after coagulation draw off the serum. Centrifuge if necessary to remove all corpuscles. The sterile serum of some other person may be used instead. More recently, sterile horse serum has been employed, but this is objectionable from the standpoint of sensitizing the patient and producing serum sickness, either as a result of subsequent intraspinal injections or whenever horse serum is administered for prophylactic or therapeutic purposes as in the administration of diphtheria and tetanus antitoxin.
2. Add to 20 c.c. of the serum $\frac{1}{50}$th to $\frac{1}{25}$th grain (0.0013 to 0.0026 gm.) of bichlorid. The smaller dose should always be employed at first and the amount increased only in subsequent injections. The dose of $\frac{1}{50}$th grain is secured by adding 1.3 c.c. of a 1:1000 solution of bichlorid in water; the $\frac{1}{25}$th grain dose is secured by adding 2.6 c.c. of the 1:1000 solution.
3. Mix and place in a water bath at 56°C. for thirty minutes.
4. Conduct spinal puncture; collect 10 to 20 c.c. of spinal fluid in the barrel of the gravity apparatus, add the mercurialized serum and inject the mixture by gravity as described on page 858.

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CHAPTER LXIV

TECHNIC OF VARIOUS METHODS FOR THE ADMINISTRATION OF BISMUTH IN THE TREATMENT OF SYPHILIS

During the past few years a very large number of new bismuth preparations have been described and advocated for the treatment of syphilis, but with few exceptions these compounds have not been subjected to thorough toxicity and spirocheticidal studies; indeed, the deplorable freedom and frequency with which new drugs are administered to human beings, before adequate preliminary investigations have been made on the lower animals, is no where better shown than in the list of arsenicals, mercurial and bismuth preparations fostered upon the medical profession by commercial laboratories during the past five years and especially in France and Germany. In a general manner it may be stated that the curative activities of all the bismuth preparations are about the same when administered on an equivalent bismuth basis, which varies from 16 per cent. in "bicreol" to 96 per cent. in "neotrepol."

At the present time bismuth is administered in the treatment of syphilis almost entirely by intramuscular injection. The advantages and disadvantages of intravenous, intramuscular and oral administration are almost identical with those pertaining to the administration of mercury discussed in the preceding chapter and for the sake of brevity these need not be reviewed with detail in this chapter.

The toxicology of bismuth has been reviewed in Chapter LV and the physician should familiarize himself with the main effects before administering bismuth in the treatment of syphilis. In a broad and general manner the toxic effects of siderosis, salivation, stomatitis, etc., are quite similar to those of mercury but usually of milder degree. The pain and nodosities of intramuscular injections of suspensions in oil are much less than follows injections of the salicylate or other mercurial salts.

Intravenous Administration of Bismuth in the Treatment of Syphilis.—Several of these new compounds of bismuth are designed for intravenous injection; some are colloidal suspensions, while others are administered in water or glucose solutions. In Germany intravenous injections of bismuth hydroxid, in colloidal suspension, in dose of 0.010 gm. (1/6th grain) for an adult, is being widely employed. I have had only a limited experience with them in experimental studies and as stated in Chapter LV the toxic effects produced by bismuth may be very much intensified as compared with intramuscular injections.

It would appear that the only indication and excuse for intravenous injections are grave cases of syphilis to whom arsphenamin and its substitutes cannot be given because of toxic reactions but which require a more active spirocheticidal drug than the soluble mercurials. I have repeatedly administered 0.01 gm. (1/6th grain) of potassium tartrobismuthate dissolved in 10 c.c. of saline solution and sterilized by heating, without ill effects; this amount is 10 to 20 times less than the usual dose by intramuscular injection and I am able to recommend it when intravenous injections are required. In rabbits a series of twelve to thirty daily injections in dose of 0.0002 gm. per kilo, which corresponds to approximately 0.01 gm. per 70 kilograms or 145 pounds, were

without discernible clinical reactions and produced no histological changes in the kidneys, liver and other internal organs; I believe, therefore, that this dose (1/6th grain) for adults is just as safe for intravenous administration in the treatment of syphilis as the bichlorid, succinimid, cyanid or other soluble mercurial compounds. My practice is to prepare the solution as follows: Dissolve 0.2 gm. of potassium tartrobismuthate in 19 c.c. of saline solution; heat at 70°C. for thirty minutes, cool and add 1 c.c. of 5 per cent. phenol. Culture and if sterile dispense in 1 c.c. amounts in 20 sterile ampules. For administration dilute 1 c.c. with 9 c.c. of saline solution in a sterile syringe and inject slowly with a No. 22 needle as described in Chapter LIX. This dose is, therefore, 0.01 gm. or 1/6th grain; phlebitis is not produced. The injections are given every two to three days for a series of ten to twelve doses.

Intramuscular Administration of Bismuth in the Treatment of Syphilis. Bismuth is almost always administered by intramuscular injections in the treatment of syphilis. Suspensions in sterile almond oil are much less painful and better borne than solutions in water or glucose.

The quartenary salt sodium and potassium tartrobismuthate occurs in two forms, the soluble and insoluble. Potassium tartrobismuthate is soluble and may be administered in solution or suspended in oil.

The dose of either compound may be 0.1 or 0.2 gm. for adults every 3 or 4 days until 2 to 3 grams have been given (20 to 30 injections of the 0.1 gm. dose or 10 to 15 injections of the 0.2 gm. dose).

Solutions may be prepared as follows: Dissolve 4 gm. of potassium tartrobismuthate or sodium and potassium tartrobismuthate in 17 c.c. of sterile saline solution or water; heat at 70°C. for sixty minutes; add 1 c.c. of 2-per cent. butyn in sterile water and 1 c.c. of 5-per cent. phenol. Culture and if sterile dispense in 1 c.c. amounts in 20 sterile ampules. Each cubic centimeter contains 0.2 gm. of drug and the butyn prevents immediate pain after injection.

Suspensions may be prepared as follows: suspend 4 gm. of potassium tartrobismuthate or sodium and potassium tartrobismuthate and 0.04 gm. of butyn in 40 c.c. of almond oil. The drugs should be gradually and thoroughly rubbed up with a small amount of oil until an even emulsion is secured when the balance of the oil may be gradually added.

Sterilize by autoclaving at 10 pounds for at least 30 minutes and culture. If sterile dispense in 2 c.c. amounts in sterile ampules which thereby contain 0.2 gm. each. Ampules with long narrow necks should not be used as the drug tends to settle in them unless constantly kept in an upright position, and require a great deal of shaking to obtain all in suspension preliminary to injection. Sterilization may be done after the ampules have been prepared instead of in bulk.

The injections are given into the gluteal muscles as described on page 841; they are almost painless.

Oral Administration of Bismuth in the Treatment of Syphilis.—As far as I am aware, bismuth has not been employed by oral administration in the treatment of syphilis. During the past two years, however, I have administered potassium tartrobismuthate and sodium tartrobismuthate internally in the treatment of chronic syphilis with tertiary skin and mucous membrane lesions, with the general result that toxic or untoward effects were not produced while the lesions were gradually dissipated. My impression, however, is that the effects were not as prompt as obtained from the internal administration of the soluble mercurials.

I have given rabbits as many as fifty consecutive daily doses of 0.02 gm. of potassium tartrobismuthate per kilo by stomach tube without producing

any histological changes in the kidneys, liver or other organs; this dose corresponded to 1.2 gm. or 20 grains per 60 kilograms and was 4 times more per body weight than given to cases of syphilis which was 0.1 gm. three times a day after meals, corresponding to 0.3 gm. or approximately 5 grains daily. The drug was dispensed both in capsules and in solution. The taste is metallic and disagreeable, but not more so than bichlorid of mercury. Potassium iodid and sodium iodid are incompatible; the former produces an immediate white precipitate while the latter forms a precipitate more slowly. The following formula is generally well borne:

Potass. tartrobismuth..... 6 gm. (ʒiss)
Aq. destil.
Syr. sarsaparil.....āā 100 c.c. (fʒvi)

One teaspoonful (approximately 2 grains or 0.12 gm.) is given three times a day after meals.

Other Methods for the Administration of Bismuth in the Treatment of Syphilis.—*Subcutaneous injections* of the soluble salts have no advantage over intramuscular injections and they usually produce more severe local reactions. Klauder has employed *inunctions* of bismuth trioxid in experimental syphilis of rabbits, but found that its spirocheticidal effects were feeble and inferior to mercury. I do not know of any other work on this route of administration. *Intraspinal injections* of bismuthized serum have not been reported upon and I have not employed them in my own practice.

CHAPTER LXV

TECHNIC OF VARIOUS METHODS FOR THE ADMINISTRATION OF THE IODIDS IN THE TREATMENT OF SYPHILIS

Since the iodids are absorbed so rapidly from the gastrointestinal canal, they are usually administered by *ingestion*. Within recent years, however, there has been an increasing tendency to administer sodium iodid by *intravenous injection*, and when a very high concentration is desired in the blood and spinal fluid for the treatment of grave cases of neurosyphilis, this method of administration has advantages. The *subcutaneous injection* of the inorganic iodids, like potassium iodid and sodium iodid, has been abandoned, because of the pain and severe local reactions produced; but combinations of iodin with oil (*iodipin*) are less painful and sometimes administered by this route. *Rectal injections* of the inorganic iodids are also employed and may be useful under certain conditions to be shortly reviewed.

Method of Ingestion. *Choice of Iodid.*—Since the iodids are absorbed so rapidly from the gastro-intestinal tract they are usually administered by ingestion. Potassium iodid is the time honored drug and was the favorite of Fournier. Hutchinson was fond of prescribing the three iodids of potassium, sodium and ammonium together in the one solution, believing that these and especially the addition of ammonium, reduced irritation. Sodium iodid is regarded by some authors as better tolerated than iodid of potassium, less irritating and less depressing. I believe it is sometimes better borne than potassium iodid, but it is, on the other hand, slightly less therapeutically active. Lipoiodin, pepto-iodin, diluted tincture of iodin, iodoform and a host of organic combinations have been advocated, but potassium iodid, sodium iodid and pepto-iodin are sufficient for the treatment of the majority

of cases of syphilis. Combinations with peptone, etc., are said to be less irritating to the stomach, probably because dissociation of the iodid ion is less rapid than in the case of the inorganic iodids; but in difficult cases my preference at present is for combinations with oil (*iodipin*) or the fatty acids, as *sajodin*, which are dissociated and eliminated less rapidly. Sajodin is also tasteless and less irritating which may be an advantage in some cases.

The syrup of ferrous iodid or the syrup of hydriodic acid may be chosen for infants, but the amounts of iodid contained are very small and I believe preference is to be given solutions of potassium or sodium iodid.

Dosage.—When first administered, small doses of the iodids are generally as effective as large doses, but sometimes a dose of 2 or 3 grains three times a day produces ill effects when 10 grains or more three times a day are better borne. Fournier states that for an adult man of good constitution and average strength, the average dose is 45 grains a day; for an adult woman in the same condition, 30 grains a day. He regarded doses smaller than these as insufficient, if not always, at least in the great majority of cases, and held that daily doses of 4 to 15 grains were quite incapable of producing the therapeutic action expected from iodid. It was his belief that the height of therapeutic activity varied from 30 to 60 grains as a daily dose, and that larger doses, while useful and indicated in certain cases, were useless in the majority. For example, in grave cases of syphilis as gumma of the palate on the verge of perforation, in neurosyphils, in severe phagedenic ulcerations, or in chronic refractory lesions, Fournier believed in large doses, but never in excess of 150 grains a day, holding that if this dose failed, so also would larger amounts.

Plan of Administration.—The iodids are of most use in the treatment of chronic syphilis, either latent or active, and I believe should be given along with intravenous injections of arsphenamin or one of its substitutes, mercury or bismuth. The effects of iodids appear to "wear off" as administration is continued, and for this reason they should be used intermittently rather than continously and in ascending doses, a maximum of 60 to 90 grains per day, however, being seldom exceeded.

It is my custom to prescribe 5 grains of potassium iodid three times a day for the first week, 10 grains three times a day for the second week and 15 grains three times a day for the third week if well borne. As a general rule, this dose need not be raised, although in some cases presenting gummas the dose is raised each week until 30 grains are being taken three times a day. I rarely exceed this dose and the administrations are kept up throughout the course of injections of arsphenamin or neoarsphenamin, etc., mercury or bismuth, which is usually twelve weeks. For the purpose of raising the dose in this manner, the usual solution of potassium iodid is prescribed:

Potass. iodidi	100.0 gm.
Aq. destil.	q.s. ad 100.0 c.c.

Each drop contains 1 grain and the patient is advised to take 5 drops (with a dropper), 10 drops, etc., as the case may be.

As a general rule, I advise taking the iodid about one half to a quarter of an hour *before* meals, *well diluted* with water, milk or ginger-ale, etc.

If signs of gastric irritation appear, it is advantageous to substitute sodium iodid in the same manner.

If a constant or fixed dose is being given and the taste is objectionable, it is advantageous to use Ricord's old prescription:

Potass. iodidi	1.5 gm.(℥iv)
Syr. corticis aurantii	200 c.c.(f℥vi)

This mixture may be given in dose of 2 teaspoonfuls (10 grains) three times a day in water or one teaspoonful (5 grains) three times a day.

Inasmuch as it is sometimes very inconvenient for a patient to take iodid three times a day, I frequently permit the whole dose of 30 to 45 grains once a day just before the main meal; if 60 grains or more per day are being taken, it is well, under these circumstances, to split the dose into two administrations rather than taking all at one time.

If signs of intolerance appear as persistent rhinorrhea, "tightness of the jaws," acne, etc., it is well to stop the administrations until these disappear and then resume with about one-half or three-fourths of the dose producing these effects.

The dose of *sajodin* (the monoiodobehenate of calcium) is 1 to 3 gm. (15–45 grains) daily in powders, as the preparation is a colorless and tasteless powder. The dose of *iodipin* is 4–8 c.c. (1–2 drs.) three times a day after meals.

Method of Intravenous Injection.—Within recent years the intravenous injection of sodium iodid has been used with increasing frequency and especially since the report by Howard[1] of its success in the treatment of a very difficult case of laryngeal syphilis to whom a series of 125 injections totalling 26,013 grains or about 3¾ avoirdupois pounds of sodium iodid had been given; Farnell[2] and others have also reported good results with this method of administration.

Indications.—As previously stated the oral administration of potassium or sodium iodid fulfills the requirements of iodid therapy in the majority of cases of syphilis, but as stated by Howard, intravenous injections are to be preferred and are indicated under certain conditions, briefly summarized as follows:

1. Whenever the full iodid effects are required, that is to say, whenever extra high concentrations are required in the blood and cerebrospinal fluid for the treatment of grave cases of tertiary syphilis as tabes, paresis, aortitis, cirrhosis of the liver, etc.
2. For the treatment of patients unable to swallow.
3. When large doses by mouth are ineffective and symptoms are progressive.
4. When signs of iodidism develop from oral administration before the desired results are obtained, that is to say, when signs of intolerance from ingestion are present, intravenous injections are sometimes well borne and deserving of trial.

The only *contra-indication* that I know of is hypersensitiveness to iodid which usually manifests itself as edema of the glottis as the most severe and dangerous lesion. Whenever possible, therefore, it is well in the case of new patients, to first administer a few doses by ingestion before intravenous injections are given, in order to make sure that hypersensitiveness is not present; fortunately, the condition is quite rare.

Dosage and Method of Administration.—Sodium iodid is to be preferred because of the low toxicity of the sodium ion. As a general rule sterile 10-per cent. solutions in water are employed. The water should be freshly distilled in order to remove bacterial and other proteins; each 10 c.c. carries 1 gram or 15 grains of iodid. The solution may be sterilized by boiling for 5 to 10 minutes without dissociation; when properly sealed, it keeps for long periods of time.

The usual dose is 1 to 2 grams (10 to 20 c.c. of 10-per cent. solution) daily or two to three times a week. The injections are given with a syringe as described on page 831. Larger doses however, may be given up to 100 c.c. or 10 grams, by means of gravity injection. Doses of this size should not be repeated oftener than twice a week except in unusually severe cases.

Combined Injections with Neoarsphenamin and Mercurials.—I sometimes inject neoarsphenamin and sodium iodid together in the same syringe twice weekly, the dose of 0.6 to 0.9 gm. of neoarsphenamin being dissolved in 5 to 10 c.c. of sterile water and mixed with 10 to 20 c.c. of the sterile 10-per cent. solution of sodium iodid. A series of twelve combined injections of this kind are given as a course (6 weeks) and at the same time sodium or potassium iodid may be given by mouth to maintain the effects of the injections.

In some cases I have also combined mercurophen in dose of 5 c.c. of 1:500 solution (1/6th grain or 0.01 gm.) or flumerin in dose of 10 c.c. of 1:50 solution (4 grains or 0.2 gm.) with 10 to 20 c.c. of 10-per cent. sodium iodid; these combinations are well borne. I have likewise given a large number of combined injections of 0.6 gm. neoarsphenamin dissolved in 10 c.c. of 10-per cent. sodium iodid solution to which is added 5 c.c. of 1:500 mercurophen or 10 c.c. of 1:50 solution of flumerin. These mixtures are slightly opalescent in the case of mercurophen, but perfectly clear in the case of flumerin and are well borne. I believe that combination therapy of this kind yields best results in the treatment of some selected cases of tertiary syphilis, but in the majority of cases the iodids are given by ingestion, while the neoarsphenamin and mercurials are administered separately.

Method of Subcutaneous Injection.—Subcutaneous injections of potassium iodid or sodium iodid are usually quite painful and large doses may produce sloughing. Intravenous injections, therefore, are very much more preferable. However, if these cannot be given and ingestion is impossible, subcutaneous injections of *iodipin* (an iodin addition product of sesame oil) may be injected in dose of 2 to 6 c.c. of 25-per cent. strength; this produces much less irritation. If the inorganic iodids are injected preference is to be given sodium iodid in dose of 1 c.c. of a 50-per cent. solution in water sterilized by boiling (0.5 gm. or about 8 grains).

Method of Rectal Injection.—This method is only required in exceptional cases when oral and intravenous administrations cannot be given. A simple cleansing enema is first given, followed by the high injection of 5 grams (77 grains) of sodium iodid or potassium iodid dissolved in 6 to 8 ounces of water to which is added 5 drops of tincture of opium. Injections are given once daily, but stopped when signs of rectal irritation develop. Larger doses are possible as up to 15 grams per day. More dilute solutions may be introduced by the drop method, but I believe the former is to be preferred when the dose does not exceed 5 grams.

Other Methods of Administration.—Lawson[3] has injected sodium iodid intraspinally, in the treatment of three cases of paresis, along with the administration of neoarsphenamin and mercury. The iodid was first given in small doses and gradually increased to 30 c.c. of a 0.6-per cent. solution (0.18 gm. or 3 grains). There was no pain or bladder disturbance, but there seemed to be an increased spasticity and temporary increase of mental confusion. There was little improvement in the spinal fluid changes, but all patients improved clinically. Excretion of the iodid appeared to be slower than when given by ingestion to normal individuals. This method, however, does not appear to be indicated in the treatment of syphilis since Osborne has shown that iodin is to be found in the cerebrospinal fluid after the ingestion of the iodids and particularly after the intravenous injection of large doses of sodium iodid. Furthermore, the only excuse for intraspinal injections in syphilis is the possibility of direct spirocheticidal effects and these small doses of iodid are certainly without this action, whereas the proteolytic or dissolving action of the iodids upon exudates and fibrous tissue can surely be better obtained by the administration of large doses by ingestion or intravenous injection.

Since the iodids are very poorly absorbed through the skin and since they are very feeble in spirocheticidal activity, it is irrational to use the liniment and ointment of potassium iodid for the treatment of syphilis.

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PART X

PROPHYLAXIS, GENERAL, SPECIFIC AND NON-SPECIFIC TREATMENT OF SYPHILIS

CHAPTER LXVI

GENERAL CONSIDERATIONS IN THE TREATMENT OF SYPHILIS

The modern treatment of syphilis includes not only the chemotherapy of this disease, but general, hygienic and other forms of treatment as well. In experimental syphilis of the lower animals we may treat the lesion or the disease, but with human subjects we must treat the patient, which requires a broad and comprehensive knowledge of general therapeutics as well as a special knowledge of syphilis. As stated by Fournier,[1] "to treat a syphilitic lesion it is sufficient to make the diagnosis and apply the remedy which experience has shown to be best under the circumstances; while the treatment of syphilis, looked upon not as a lesion, but as a disease, is much more comprehensive and complex. The latter includes a number of most different questions, all difficult, controvertible and controverted. It even includes general questions of major importance, on which may depend the success or failure of treatment." Again "to treat syphilis is not only to attack and cure the manifestations of a day, or even of a period; it is also and especially to attack the disease as a whole, to attack it when patent and latent; to lay hold of its primary cause, the origin of its actual and future determinations. It is to institute, with this object, a treatment of long duration—almost chronic—which alone is sufficient to realize *preventive* effects—that is, to protect the patient against the future, and to safeguard not only himself, but his posterity."

Since these words were written by the great French syphilologist, epoch making discoveries during the past twenty years have confirmed their truth and especially in the treatment of chronic syphilis. Indeed, Fournier did not live long enough to realize their full significance, since the discovery of *Spirochæta pallida*, along with methods for its detection in the tissues and with the discovery of the complement-fixation reaction, has shown how widespread the infection may become in the tissues and organs of individual cases and how difficult it may be to bring about cure in the sense of complete destruction of the parasites. Fournier clearly realized and taught that the dissipation of obvious lesions did not necessarily mean the cure of the disease and modern discoveries have amply confirmed his clinical opinions.

Modern Treatment of Syphilis an Example for Future Chemotherapeutic Investigation.—Our knowledge of syphilis is still far from complete, but in no other disease known to mankind have such wonderful discoveries been made in the etiology, pathology, immunity and treatment as have been made in syphilis during the past twenty years. Its cause, *Spirochæta pallida*, was

discovered by Schaudinn in 1905 and this was followed within a short time by the development of methods for the detection of the parasite in the secretions of the chancre, thereby opening up methods for the immediate diagnosis and treatment of the disease in its earliest stage instead of waiting for confirmation by the appearance of the secondary lesions of generalized infection. Within a year after this epoch-making discovery, the first application of Bordet's and Gengou's phenomenon of complement fixation for the diagnosis of disease was made by Wassermann and his colleagues and independently by Detre, in the diagnosis of tuberculosis and syphilis, and since then this serological reaction has been developed into a diagnostic method for syphilis without parallel today as a practical and valuable procedure for the diagnosis of disease and as a means for the control or check upon its treatment. And just about four years later Ehrlich, Bertheim and Hata discovered salvarsan for the treatment of syphilis in a series of researches without comparison in brilliance and fruitful results which put the climax upon this period of remarkable discoveries stretching over a period of but little more than five years from the time of Schaudinn's discovery. As previously stated this remarkable triad of discoveries is without parallel in the history of medicine and since then numerous other discoveries along the paths blazed by Schaudinn, Bordet and Wassermann, Ehrlich and Hata have rendered the diagnosis and treatment of syphilis a lasting testimonial and monument to what may be accomplished by prepared minds and systematic research.

The modern treatment of syphilis will stand as a goal and ideal for future efforts in chemotherapeutic research. The chemotherapy of this disease is a perfect embodiment of the underlying principles and difficulties of research in this field of the art and science of medicine. It has shown us that to prepare new compounds, determine their toxicity for healthy animals and finally their curative activities for an experimentally produced infection, is not sufficient; we must know something of their absorption and elimination, their toxic effects in the diseased human subject and finally their curative activity as modified by the pathological and immunological changes of disease. The modern treatment of syphilis, and especially of chronic syphilis, is not yet upon a satisfactory basis; more, much more, must be discovered for the successful treatment of chronic syphilis although the treatment of early syphilis by modern chemotherapeutic measures may be regarded as practically a solved problem.

I may repeat, therefore, that the phenomenal progress made in the treatment of syphilis during the past decade has blazed the trail for similar efforts in other diseases and has shown how complicated and laborious may be chemotherapeutic research even under the most favorable circumstances. Experience in the treatment of human syphilis has shown that many and important factors require consideration and solution after the fundamental facts have been established by animal experimentation. And, in passing, it is fitting to pay tribute to the enormous value of animal experimentation in the development of the modern treatment of syphilis, trypanosomiasis and other diseases; a mere glance at the preceeding chapters, summarizing the results of such investigations, is sufficient for indicating the debt we owe legitimate and systematized experiments upon the lower animals. Without animal experimentation salvarsan would not have been discovered and without it future progress in the chemotherapy of disease would not be possible.

Regarding the Standardization of the Treatment of Syphilis.—For several hundred years attempts have been made to standardize, as it were, the treatment of syphilis with mercury or a combination of mercury and the

iodids. Since the discovery of salvarsan and allied compounds, attempts in this direction have been renewed and the medical profession in general has ever been ready to welcome any clear cut and brief directions generally applicable for the treatment of all cases.

But such is not advisable, except possibly in the treatment of early syphilis. We may readily enough standardize a laboratory test but not the treatment of a disease, because of the possibility and probability of so many modifying factors requiring consideration and the necessity for treating the patient rather than a symptom, a lesion or the disease itself. Probably in syphilis we may go further; it is possible to lay down a plan of treatment for the medicinal prophylaxis of the disease generally applicable to all cases and we can express an opinion concerning the minimum amount of treatment for all or, at least, the majority of cases during the first six to twelve months after the appearance of the chancre. The majority of cases of primary syphilis are otherwise healthy individuals, that is, there is not usually present some other disease of sufficient severity to materially alter or influence our set plan of treatment for early syphilis. Likewise we may adhere to a set plan of general treatment, as the search for focal infection with special reference to the teeth and gums, the correction of dietary and hygienic errors, etc. But after the first six to twelve months we should individualize our patients and especially all cases coming under observation in the late stages of the disease regardless of the kind and amount of treatment previously received. Therefore, while we may be justified in expressing an opinion on what should constitute a plan of treatment generally applicable to all cases of syphilis during the first six to twelve months after infection and on what should constitute a minimum amount of treatment at this time, regardless of negative Wassermann reactions and freedom from clinical signs and symptoms, we can hardly generalize treatment beyond this period. For late cases of syphilis we may reach a decision upon what constitutes a "course of treatment" and when an organic arsenical, mercury, bismuth or an iodid compound is indicated singly or in combination, but the size and frequency of the dose, route of administration, number of "courses," intervals of rest from specific medication, general treatment, etc., are factors requiring more or less arrangement for individual cases. If it were possible to eradicate all *Spirochæta pallida* from the tissues of all cases of syphilis with a medicinal compound free and safe from untoward toxic effects, nothing would be easier than the treatment of the disease by rule of thumb. In the first year after the discovery of salvarsan, hopes of this kind were entertained because of its successful accomplishment in experimental infections of the lower animals. But we know to-day that there is no such medicinal available at present; in the treatment of frambesia and very early syphilis with arsphenamin or neoarsphenamin, *magna therapie sterilsans* has come nearest to accomplishment in human disease, but even in these we know that one dose is not usually sufficient and that the administration of repeated doses may be complicated or indeed rendered impossible by unexpected toxic effects. At best we can but realize that we have in some of the organic arsenical compounds and in some of the salts of bismuth and mercury powerful spirocheticidal medicaments, but that their administration must be guided by our present knowledge of their absorption and elimination and their pharmacological and toxicological effects in *sick* human subjects as well as in normal lower animals. Furthermore, we must possess a sound working knowledge of the pathology of syphilis and the modifying influence upon treatment that may be exerted by diseases with which an individual patient may be struggling in addition to syphilis.

By reason of these widespread influences the treatment of syphilis is becoming a specialty in medicine, but owing to its prevalence the disease is encountered in all fields of medicine and surgery and belongs to no one specialty. Indeed, the complete and detailed diagnosis of chronic syphilis may require a very wide training in dermatology, neurology, psychiatry, opthalmology, rhino-laryngology, surgery and internal medicine. It is unwise to treat a lesion; the ophthalmologist, for example, may plan treatment for an interstitial keratitis or an optic atrophy and entirely overlook lesions elsewhere. I believe that the treatment of syphilis may properly become a specialty in medicine by reason of the special training and experience required for the treatment of the disease as a whole and particularly of the central nervous system, but its diagnosis belongs to no one specialty and frequently requires the cooperation of physicians highly experienced in several specialties.

Constitution as a Factor in the Treatment of Syphilis.—In other words, the intelligent and effective treatment of syphilis requires a thorough working knowledge of the principles underlying the mechanism of infection and the pathogenesis of the disease together with a knowledge of the immunological changes that occur, as, likewise, the pathological alterations that may be produced in different tissues and organs and the clinical and laboratory means for their detection. Furthermore, it is apparent that the constitution of different individuals influences the course of the disease and the amount of treatment required for its eradication or arrest. As stated by Schindler[2] for example, a man still in the secondary stage of syphilis required ten times as much treatment to change his Wassermann reaction to negative as did his wife, infected by him and also in the secondary stage. If the husband's spirochetes were arsenic-fast, why did they not retain this resistance in the body of the wife? Schindler believes that it is not a difference in strains of spirochetes but rather a different ability on the part of the patient's constitution to react to the infection and treatment. As recently stated by Finger[3] the clinical picture of syphilis has changed during the past two centuries with the result that the cutaneous lesions are becoming milder but with an increase of syphilitic aortitis and involvements of the central nervous system. It is likely that a part of this change is influenced by constitution and the present day strenuous habits of living which tend to increase injuries of the cardiovascular and nervous systems producing areas of lowered resistance and favoring localization of spirochetes.

Importance of Fundamental Principles in the Treatment of Syphilis.—The treatment of syphilis demands, therefore, a sound working knowledge of the underlying principles of the mechanism of infection and pathology of the disease, the rôle of acquired immunity, as well as a knowledge of the comparative curative properties of various antisyphilitic remedies including their pharmacological and toxicological effects. Unfortunately, however, there is too great a tendency to treat syphilis by set rules which is always apt to be the case when specific remedies are available for the treatment of diseases; this may result in too little treatment for most cases, too much for some and meddlesome or harmful therapy for others.

Some physicians still adhere to the belief that syphilis may be aborted by cauterization or excision of the chancre. For several hundreds of years this practice has had its adherents and many successes have been claimed, but Fournier's remarkably clear analysis of the situation from the clinical standpoint, together with his large experience, shows quite clearly that success can hardly be expected if the sore is actually a syphilitic chancre. As he points out not a few of the alleged successes have been due to the cauterization or

excision of "simple chancres," that is, non-syphilitic abrasions, ulcers or herpetiform lesions, with an incubation period of only one to twelve days after exposure and infection. Since the discovery of *Spirochæta pallida*, Neisser, Brown and Pearce and others have shown, in experimental infections of monkeys and rabbits, that the organisms may reach the neighboring lymphatic glands within a few days after infection and many days or weeks before the development of the chancre. Undoubtedly syphilis may be prevented in some cases at least by the thorough application of soap and water, calomel ointment or some other spirocheticidal substance within an hour or two after exposure, but if one or two days have elapsed, abortion of the disease requires the administration of arsphenamin or an allied compound. As Fournier has stated, "*cauterization of a syphilitic chancre, even in its earliest stage, is absolutely powerless to prevent constitutional infection.*" The same is true of excision. Yet we quite commonly hear of physicians "cauterizing" penile sores and claiming thereby to abort syphilis; every once in a while we see cases of chronic syphilis who have relied upon this procedure alone and are dumbfounded to learn of its failure.

The rapid generalization of infection with *Spirochæta pallida* by the lymphatic and blood streams after the appearance of the chancre, is not sufficiently realized and within the last few years has lead to the more frequent examination of the spinal fluid for evidences of neural invasion even during the early stages of the disease. The old idea that infection of the central nervous system is only a late accident in syphilis should be abandoned and spinal puncture, with careful cytological, chemical and immunological examinations of the cerebrospinal fluid, resorted to within the first year after contraction of the disease, to determine whether or not these important tissues have been invaded and infected and treatment given accordingly.

In this connection the question of specific tissue strains of *Spirochæta pallida* has commanded a great deal of attention within the last few years and especially with reference to the existence of a specific strain for the brain and spinal cord, the so-called "neurotropic strain." The evidence for and against this hypothesis has been reviewed in Chapter XXXVI, but here it may be mentioned that no evidence at all is furnished by the size and appearance of the chancre or secondary lesions. It is commonly stated that the "neurotropic strain" produces a small erosive type of chancre with poorly developed secondary skin lesions, while the less dangerous "dermotropic strain" produces a large chancre and florid secondaries. But clinical experience does not substantiate this claim. It is true that in some families neurosyphilis develops so early and so frequently as to lend weight to the supposition that a specific neurotropic strain of *Spirocheta pallida* exists in the production of paresis and tabes, but such may be explained on the basis of inherited factors of predisposition and the results of experiments with rabbits upon this question of specific strains have been seriously questioned by the possibility of confusion with the organism of rabbit spirochetosis—*Spirochæta cuniculi.*

A common error in the treatment of chronic syphilis is to expect the total disappearance of all lesions along with a complete disappearance of all symptoms and functional defects. It is true that with treatment syphilitic exudates and fibrous tissues may become absorbed with at least partial restoration of function, but when a tissue has been completely destroyed as important nerve cells or tracts, restoration of function cannot be expected even if it were possible to prevent further destruction. Furthermore a mental picture of the pathological changes produced by chronic infection with *Spirochæta pallida* aids greatly in a proper understanding of the mechanism of medication and the necessity for administrating compounds in such

manner as will best favor gradual penetration of the inflammatory tissues in which the parasites are apt to be imbedded.

Furthermore, in connection with this subject of the influence of pathology upon treatment and prognosis, it should not be forgotten that the co-existence of some other non-syphilitic disease may require due consideration in our therapeutic efforts. For example, leprosy in a syphilitic is sometimes rendered worse by the administration of potassium iodid which favors renewed activity of the leprosy bacilli; the same is sometimes encountered in tuberculosis.

And few subjects in syphilis are as little understood as the immunological changes in this disease. To-day it is hardly necessary to raise the question as did Fournier in his treatise upon the treatment of syphilis, whether it is necessary to treat every case of syphilis. No better answer to this question can be found anywhere in literature than contained in the first two chapters of his book, only to-day we have the advantage of a deeper knowledge of the etiology and the results of immunological discoveries and investigations in experimental and human syphilis for substantiating his claim that all cases require treatment. That is to say, while a very exceptional case of syphilis may completely recover by the operation of his own cellular and humoral immunological agencies, this can never be depended upon; that there is "no criterion by which we can estimate the quality of early syphilis and its future progress" and that "in the case of syphilis the present is in no way the mirror of the future."

Immunity certainly does exert an important rôle in the fate of the syphilitic and particularly in the part it plays in protecting organs against progressive infection with *Spirochæta pallida;* likewise in the resistance offered superinfection and re-infection in syphilis. Few subjects in this disease are more interesting and likewise more important in relation to diagnosis, prognosis and even of specific and general treatment, but these subjects have been already discussed in Chapter XXXVII.

From the standpoint of serological changes in relation to diagnosis, treatment and prognosis of syphilis, mention need only be made of the complement-fixation reaction with serum and the colloidal gold reaction with spinal fluid. It is my conviction that the complement-fixation reaction in syphilis is without parallel in medicine as a valuable aid in diagnosis, when a few simple principles concerning its limitations are understood and properly evaluated. The test is not at all intricate for properly trained and experienced workers; certainly it offers chances for technical errors as is true of all laboratory tests, even the simplest, but technical errors can be easily reduced to a negligible minimum and biological errors may occur only in frambesia and trypanosomiasis. With a technically acceptable method the physician need not worry so much about the possibility of falsely positive reactions, but rather of falsely negative reactions. Altogether too frequently physicians depend upon the serum test for aiding the diagnosis of the chancre, when it should be realized that the reaction may be negative at this stage since sufficient time may not have elapsed for the production of the so-called "reagin" or complement-fixing antibody-like substance. The importance of early diagnosis and prompt treatment is still too frequently overlooked and in the majority of cases of primary syphilis the diagnosis may be made or greatly aided by the direct microscopical examination of the secretions of the chancre for *Spirochæta pallida* by appropriate staining methods or better, by means of a dark-field microscopic examination.

Furthermore, too much weight is ordinarily placed upon a single negative reaction in excluding the diagnosis of latent syphilis, when the pathology of syphilis should be remembered to the extent that *Spirochæta pallida* may be

so greatly reduced in virulence as to produce little or no tissue reaction and consequently too little "reagin" in the serum and spinal fluid for detection in the test-tube. The Bordet-Wassermann test is an invaluable aid in diagnosis and as a control on treatment, but only when it is properly understood and employed. Great harm is being constantly done by the blind use of the test—both by those who place too much confidence in it and by those who have no confidence in it at all.

Like so many subjects in medicine there is a valuable middle ground; if serologists will only realize their responsibility in conducting the test in a technically correct and acceptable manner and if clinicians will only take enough time for acquiring a sound working knowledge of its advantages and limitations and honestly admit that their clinical opinions and diagnoses are not infallible, the test is of incalculable value for aiding the diagnosis of syphilis after the primary stage and, under certain conditions, of value as a check in the treatment of the disease.

And in the actual treatment of syphilis let us properly differentiate between the treatment of early and chronic syphilis, because conditions are not the same in both. In the former the infection has not brought about the profound tissue changes which in the latter may influence our choice of drugs, their dose and manner of administration. We must have a proper understanding of the relative spirocheticidal properties of various medicaments, their absorption and elimination as well as their toxicological effects. We must resolutely refuse to adopt into our therapeutic armamentarium drugs exploited for commercial reasons only, until sufficient and appropriate laboratory studies have been made to warrant their use in the treatment of human syphilis possibly to the exclusion of compounds of proven therapeutic value. Let us realize that it is possible by animal experimentation to obtain a very accurate gauge of the spirocheticidal activity and toxicity of any compound proposed for the treatment of syphilis and let us refuse to use any compound unless such investigations have been made by responsible individuals. Of course some medicaments are useful in syphilis even though lacking in direct spirocheticidal activity; the iodids, for example, are not greatly spirocheticidal in the doses ordinarily administered, but nevertheless are of aid in the treatment of chronic syphilis. But let no one delude himself into believing that the iodids alone will cure syphilis; not infrequently we meet with cases of chronic syphilis to whom physicians have administered nothing but potassium iodid even in the early stages. Of course, the measure of the spirocheticidal activity of a medicament may not be an estimate of its sole therapeutic power; sodium cacodylate, for example, may favorably influence a syphilitic lesion but its curative power is so much less than arsphenamin, neoarsphenamin and allied compounds that it would appear to be a waste of time to administer it and especially since its curative properties in experimental syphilis are known to be almost negligible. Such things as intramuscular injections of milk or other forms of non-specific protein therapy may aid in the treatment of syphilis as they may in some chronic bacterial infections, but it is imperative for physicians to have a sound working knowledge of what each drug is known to do or capable of doing in the treatment of syphilis, before he is justified in employing it. In general terms, medicinal compounds known to exert the most marked degrees of spirocheticidal effects in experimental syphilis of the lower animals, likewise prove most spirocheticidal and effective in syphilis of human beings and should be preferred in the treatment of syphilis providing this is possible from the standpoint of administration by an acceptable route and freedom from both immediate and late toxicological effects.

Finally it cannot be too strongly emphasized that all compounds known to-day as possessing spirocheticidal activity and curative properties in syphilis are capable of exerting toxicological effects and doing actual harm. I believe it is far more important to understand what these effects may be, and how to detect them by clinical and laboratory means, than to attempt defining dosage according to set rules. It must never be forgotten that susceptibility to the toxic effects of the drugs employed in the treatment of syphilis varies considerably among individuals and is probably influenced by the pathological changes produced by syphilitic or non-syphilitic infection. A knowledge of these immediate and delayed or late untoward effects, including the possibility of stimulating or provoking syphilitic infection into renewed activity by well meant therapeutic measures, will aid in preventing our falling into the error of meddlesome therapy and to be content to leave well enough alone under certain circumstances. Altogether too frequently a diagnosis of syphilis is taken as a signal for rushing in with therapeutic measures that may do more harm than good even though our drugs are known to be spirocheticidal agents and are properly administered.

I believe, therefore, that in any treatise upon the treatment of syphilis that more space should be devoted to a frank discussion of these and other fundamental principles than an endeavor to lay down formal and short cut rules for treatment in this or that stage. The latter are useful as guides for the treatment of typical cases, but syphilis is a disease capable of involving so many different organs which may modify any course of treatment, the medicaments employed are so powerful and capable of doing much harm as well as much good, the disease requires such prolonged treatment and good judgment, that it is folly to attempt describing its adequate treatment in a brief way or formulating therapeutic rules and regulations for all cases.

In the preceding chapters I have, therefore, discussed more completely these fundamental principles in relation to the treatment of syphilis and will now take up the actual treatment of the disease in its early and late stages.

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CHAPTER LXVII

COMPARATIVE ADVANTAGES AND DISADVANTAGES OF THE ORGANIC ARSENICALS, MERCURIALS, BISMUTH AND IODID COMPOUNDS IN THE TREATMENT OF SYPHILIS

So many new compounds have been placed before the medical profession during the past twelve years for the specific treatment of syphilis that a great deal of confusion regarding their comparative advantages and disadvantages exists in the minds of not a few physicians called upon to treat the disease. Numerous questions frequently arise of which the following are examples: (1) How do arsphenamin, neoarsphenamin, silver arsphenamin, sulphar-

sphenamin, etc., compare in curative activity? (2) Are the American made arsphenamins as efficient as the original German products? (3) Have the bismuth compounds been proven curative in syphilis? (4) How do bismuth and mercurial compounds compare in curative activity? (5) Are there special indications for the arsphenamins in some cases, for mercury and bismuth in others, etc.? (6) Can the arsphenamins alone or bismuth alone, etc., be depended upon for the treatment of syphilis? These and a host of similar questions are constantly being raised and unfortunately final answers cannot yet be given to some of them. The best one can do is to seek answers from two main sources, namely, (1) the results of experiments in the treatment of syphilis of the lower animals where the conditions are such, under proper and acceptable conditions, that comparisons can be made with a fair degree of scientific accuracy and (2) the results of clinical experience. The latter is, of course, the more important of the two, but unfortunately the present day remedies and methods of treating syphilis cannot be evaluated in definite and final terms; one or more decades must elapse for "time" to tell the final story, but it is possible to describe the results being observed and to use these as a basis for formulating opinions. In this chapter I shall endeavor to formulate answers to some of these questions on the basis primarily of my own experiments and experience, together with the published observations of others; obviously it is inadvisable in a treatise of this kind to attempt a review of all of the available literature even if this were possible. After all, statistical reviews are usually very inaccurate and only broad and general deductions and conclusions are justifiable because syphilis as a disease manifests itself in many different ways in different individuals with many modifying conditions and influences and it is so easy to dissipate the obvious lesions and relieve symptoms with the drawing of hasty conclusions that do not stand the test of time.

Specific Treatment of Syphilis.—In the first place, what constitutes the specific treatment of syphilis? As Fournier has stated the word "specific" is one of those which every one interprets in his own way. The only correct definition is one that ascribes specificity to those medicinal compounds which exert a direct and peculiar destructive effect upon the parasite of the disease. But these effects may not occur in some cases; the word "specificity," therefore, is not synonymous with curative "infallibility." We are too prone to regard a specific remedy or specific treatment as unfailing; the remedy may be a specific in the true sense of the term and yet fail to even arrest the progress of a syphilitic infection, as, for example, in some cases of paresis. The parasites may possess a peculiar increased resistance to the destructive effects of the drug or the drug may not be able to enter into contact with the parasites in sufficient concentration to destroy them; these and other factors which we do not understand at present may nullify the specific activities of a particular compound even when administered in a most thorough and acceptable manner. But unquestionably the arsphenamins are less likely to fail than mercury, although they may fail just as quinin may fail in the treatment of malaria and as opium may fail to relieve some pains.

Comparative Spirocheticidal Activity of Specifics in Acute Syphilis of Rabbits.—With these few remarks on specific medication in syphilis, I may now pass on to a more detailed discussion of the spirocheticidal activities of the remedies regarded as exerting specific effects. For the purpose of comparing them in these respects, experiments in the treatment of rabbit syphilis have proven of some value, because the final results can be obtained within a year whereas in human beings many years must elapse and, indeed, the final results are ascertainable in only a very small percentage of cases.

The curative activity of any drug in syphilis of the rabbit or human being is probably dependent not only upon its direct spirocheticidal activity when once in union with the parasites, but likewise upon penetrability of syphilitic exudates and the degree of stimulation of antibody production. The latter does not refer to the engendering of antibodies by the medicament itself; as stated in Chapter XXXVII, there is little or no evidence to support the hypothesis that such occurs in either the human being or lower animal, but medicaments probably vary in their power of releasing antigenic substances from spirochetes which in turn engender some antibody production. Probably antibody production under these circumstances bears a relation to spirocheticidal activity, but there is no evidence available upon which to base an opinion.

Under ordinary circumstances syphilis of the rabbit is much milder than syphilis of the human being although as stated in Chapter XXI, suppression of the primary lesion sometimes results in the widespread distribution of *Spirochæta pallida* with the production of lesions of the skin and mucous membranes, lymph-glands, bones, special organs, etc., similar to the lesions of syphilis in the human being. But natural immunity in the rabbit to *Spirochæta pallida* is more marked than in human beings; likewise, the immunity response in untreated and treated syphilis of this animal is more decided and these factors render the cure of syphilis of the rabbits much easier of accomplishment than syphilis of human beings. Nevertheless experiments in the chemotherapy of rabbit syphilis have yielded data of much value regarding the curative activities of various medicaments and the size, frequency, and route of their administration, just as valuable data of a similar nature have been obtained in the chemotherapy of experimental trypanosomiasis of this animal for application in the treatment of trypanosome diseases of human beings.

Comparison of the Curative Activity of Various Medicaments in Acute Primary Testicular Syphilis of Rabbits.—In Chapters XXIII to XXIX I have described in detail the curative properties of a large number of different compounds in experimental syphilis of the rabbit; not all are suitable or worthy for use in the treatment of human syphilis. In the following table the better known and more useful of these are listed along with the maximum tolerated and minimum curative doses of each as determined in my laboratory for purposes of comparison. As previously stated in Chapter XLIV, the maximum tolerated dose of any medicament for the rabbit has only a broad and general relation to its toxicity for the human being. In the latter, we may have hypersensitiveness and minor reactions develop after the administration of much smaller doses according to body weight, but in a general manner compounds best tolerated by the rabbit are probably best tolerated by human beings. The minimal curative doses listed in table 6 are average amounts capable not only of dissipating the local testicular or scrotal lesions but effecting complete sterilization of the lymphatic glands and thereby permanent cures:

TABLE 6.—COMPARISON OF THE CURATIVE PROPERTIES OF SINGLE DOSES OF VARIOUS MEDICAMENTS IN TESTICULAR SYPHILIS OF THE RABBIT

COMPOUNDS	MAXIMUM TOLERATED DOSE PER KILO (GMS.)	MINIMAL CURATIVE DOSE PER KILO (GMS.)	CHEMOTHERAPEUTIC INDICES
Neoarsphenamin	0.350	0.020	1:18
Sulpharsphenamin	0.300	0.022	1:14
Silver arsphenamin	0.110	0.010	1:11
Arsphenamin	0.120	0.012	1:10
Sodium arsphenamin	0.210	0.020	1:10
Galyl	0.100	0.020	1:5
Tryparsamid	0.900	0.400	1:2
Sodium and potassium tartro-bismuthate	0.200	0.050	1:4
Mercuric chlorid	0.003	0.003	1:1
Salicylate mercury	0.005	0.005	1:1
Flumerin	0.030	0.030	1:1
Potassium iodid	0.310	0.300	0

The chemotherapeutic indices show in a general manner the comparative spirocheticidal activities of these various compounds employed in the treatment of syphilis. Unquestionably arsphenamin and its substitutes stand at the head of the list followed in order by the pentavalent arsenicals, bismuth, mercury and the iodids. If the *chemotherapeutic indices* may be used for expressing comparative spirocheticidal effects, these various compounds may be listed as follows:

Arsphenamin, neoarsphenamin, sulpharsphenamin and other trivalent organic arsenicals	10 to 18
Tryparsamid and other pentavalent organic arsenicals	2 to 6
Bismuth	4 to 5
Mercury	1
Iodids	0 to 1

These figures are based upon single doses for rabbits, sufficient for complete sterilization including the lymph-glands and not merely for the dissipation of local testicular or scrotal lesions alone; the amounts required to merely cause the disappearance of spirochetes and healing of the local lesions are much smaller.

Comparative Spirocheticidal Activity of Specifics in Human Syphilis. This naturally brings one to the consideration of a more important practical question, namely, the comparative specific spirocheticidal activity of the large number of arsenical, mercurial, bismuth and iodic compounds employed in the treatment of human syphilis. Here our difficulties are infinitely greater than in syphilis of the lower animals because it is almost impossible to compare one remedy with another under strictly acceptable conditions because of variations in the virulence and duration of infection, the influence of occupation, general health, immunological response, sex, etc. In rabbits we may work with a single strain of *Sp. pallida*, use animals of the same breed and stock, approximately of the same weight and general health, and infect a large series at the same time, choosing for our tests, animals presenting similar lesions and treating each strictly according to body weight. We may keep them under constant observation and make examinations for spirochetes as frequently as we choose, at stated intervals and finally judge the results by necropsies and the inoculation of fresh animals with emulsions of the lymph-glands and other organs of the treated animals for surviving spirochetes. It is not possible however, to duplicate these conditions in human beings. We may estimate the time required for the removal of spirochetes from chancres and mucous patches and for the dissipation of these and other obvious lesions but we cannot ordinarily give our drugs strictly according to body weight and we cannot secure enough cases showing similar lesions of approximately the same duration and extent, etc., as are so easily obtained in rabbit syphilis. Therefore, we must resort to animal experimentation for data of real value although this data is applicable and referable only to acute syphilis. In chronic syphilis the location of the spirochetes, the degree of tissue reaction about them, etc., are factors greatly modifying our conception of the comparative values of specific spirocheticidal remedies. But here again rabbit syphilis yields information of value since it is possible to produce chronic lesions by infecting and castrating large numbers and selecting animals presenting approximately the same lesions for our tests.

But if we adhere closely to our theme we may express in comparative terms the specific spirocheticidal activities of the various remedies employed in the treatment of human syphilis. These, however, are only a small part of the practical value of the respective compounds; one must keep in mind their pharmacological and toxicological effects, their penetrability of syphilitic exudates, etc., for properly expressing a complete estimate of their value in syphilis. But leaving out for the present these important modifying factors, I believe that the comparative spirocheticidal properties of various remedies used at present in the treatment of syphilis of human beings may be arranged in the following order:

Group I. Arsphenamin and silver arsphenamin highest and about equal.

Group II. Neoarsphenamin, sulpharsphenamin, sodium arsphenamin and silver neoarsphenamin next and about equal; spirocheticidal activities

about two-thirds that of Group I but varying considerably, and especially in the case of neoarsphenamin, according to the brand employed.

Group III. The bismuth compounds next; spirocheticidal activity about one-fifth that of Group I but varying considerably according to the compound employed.

Group IV. Tryparsamid, sodium oxyaminophenyl arsenate ("189"), amino-arsenophenol (Pomaret's "132"), "galyl" and other pentavalent arsenicals next; spirocheticidal activity about one-sixth that of Group I.

Group V. The mercurial compounds varying greatly in therapeutic activity according to content of mercury, rapidity of dissociation and ionization of mercury, solubility, etc.

This classification is based partly upon the results of experiments in the treatment of rabbit syphilis and more especially in the treatment of acute syphilis of human beings as observed personally or gathered from the literature. In a broad and general manner, I believe it answers the question concerning the comparative specific spirocheticidal activities of the respective medicaments. It does not, however, necessarily indicate the order of choice in the treatment of syphilis; tryparsamid for example, is very much less spirocheticidal than arsphenamin, but is said to be more efficacious in the treatment of paresis, partly on the basis of claims that it better penetrates the tissues of the central nervous system. It is necessary, therefore, to discuss the respective compounds more broadly before arriving at an estimate of their practical value; in other words, one compound may be better than another for a certain clinical type of syphilis even though it is less spirocheticidal in acute syphilitic lesions of rabbits or human beings.

Advantages and Disadvantages of Arsphenamin in the Treatment of Syphilis.—What is the present status of arsphenamin or salvarsan in the treatment of syphilis? Fifteen years have now elapsed since it was discovered and introduced for the treatment of syphilis by Ehrlich, Hata and Bertheim and an enormous literature has accumulated upon its use in the treatment of this and other protozoan diseases. As stated above it is still too early to express a final opinion of its value in the cure of syphilis because only time can tell the final story in terms of the incidence of late tertiary syphilis of the cardiovascular and central nervous systems of individuals treated in the early stages and regarded as "cured" according to our present day criteria. But I believe the results of laboratory and clinical studies warrant the following statements at the present time:

1. Arsphenamin or salvarsan is the best spirocheticide in the body known to-day. As will be shortly discussed, silver arsphenamin is somewhat more spirocheticidal in rabbit syphilis but in the treatment of human syphilis the slight differences are not usually apparent in the majority of cases. Within the last six or seven years neoarsphenamin has gradually superceded arsphenamin in the treatment of syphilis, because of its lower toxicity and greater ease of administration, but the fact remains that it is usually inferior in spirocheticidal activity. It is commonly stated that 0.9 gm. of neoarsphenamin is equal in curative activity to 0.6 gm. of arsphenamin; this is true insofar as arsenic content is concerned, but in my experience slightly more than this amount of neoarsphenamin is required to equal arsphenamin which I believe is largely because neoarsphenamin is eliminated more rapidly than arsphenamin. I see no escape from the conclusion that, judged solely on the basis of spirocheticidal activity and specific medication, arsphenamin is the sovereign remedy for syphilis to-day.

2. Arsphenamin is the most stable of the organic trivalent arsenicals. As will be shortly discussed, neoarsphenamin, sulpharsphenamin and sodium

arsphenamin may vary considerably in spirocheticidal activities as prepared in different laboratories and even in the same laboratory, but arsphenamin remains remarkably constant; and this is a point of considerable importance in favor of arsphenamin, because when the physician employs it in the treatment of syphilis, one can be sure of spirocheticidal effects whereas with the neoarsphenamins this is not always the case. From the standpoint of spirocheticidal activity the arsphenamins prepared in this country have been practically identical with the original German salvarsan. One sometimes hears it stated that the clinical results observed with the original German salvarsan between 1912 to 1916 were superior to those observed since 1916 with American, English and French products; I am not able to discuss the question from the standpoint of the English and French arsphenamins because of a lack of experience with them, but as far as I am able to judge clinically and more especially by laboratory studies, American made arsphenamins are equal on an average basis to the original German arsphenamin manufactured in the years from 1912 to 1916. It is very easy to be mislead by first impressions and the word "original" has a strong appeal to many minds as applied to the German arsphenamin or salvarsan prepared by Ehrlich's method, but I can only repeat that it is my experience that the original salvarsan was not superior in spirocheticidal activity to present day American made arsphenamin. It is true that some lots have proven more trypanocidal and spirocheticidal than others, but we cannot make our tests mathematically correct and the *average* parasiticidal effects of American arsphenamin equals the *average* effects of German salvarsan.

3. Arsphenamin is more slowly eliminated than neoarsphenamin and sulpharsphenamin. This is probably because it is precipitated at the hydroxyl ion concentration of the blood with the result that it is fixed by the tissues and is eliminated more slowly, but for these reasons it would appear better adapted for the treatmet of chronic syphilis than neoarsphenamin and sulpharsphenamin. This is especially true of the treatment of neurosyphilis since it appears that arsphenamin penetrates the tissues of the brain and cord better than neoarsphenamin. On the other hand, cases of chronic syphilis are more likely to develop toxic accidents because of focal infections and various pathological changes in the organs of elimination and, it is possible, that arsphenamin is more dangerous than neoarsphenamin in the vascular types of neurosyphilis because of its greater vasculo-toxicity.

4. Arsphenamin is undoubtedly more toxic than neoarsphenamin and sulpharsphenamin. It is much more likely to produce immediate and early toxic reactions because of colloidal reactions in the blood as well as by its greater vasculo-toxicity. I believe it is more likely to produce dermatitis and allied accidents than neoarsphenamin but less so than sulpharsphenamin; it is also more likely to produce jaundice because more hepatoxic and longer retained in the liver than neoarsphenamin. It is also more likely to produce neural and cardiovascular Herxheimer reactions because of its greater vasculo-toxicity and spirocheticidal properties.

5. Arsphenamin is more difficult to administer than neoarsphenamin and sulpharsphenamin. Many fatalities have been caused by failure to neutralize or alkalinize the solutions. It tends to produce greater irritation of the veins. It is much more difficult to give a series of injections than is the case with neoarsphenamin and sulpharsphenamin. Because of these technical difficulties and toxic effects, patients are less likely to take an adequate number of injections and not a few physicians are likely to undertreat their cases. Undoubtedly neoarsphenamin and sulpharsphenamin have induced a larger number of physicians to undertake the treatment of syphilis and have

encouraged the more thorough treatment of the disease by both physician and patient.

To sum up, it may be stated that arsphenamin is the best spirocheticide known to-day for the specific treatment of syphilis; it is the arsenical of choice in the treatment of both acute and chronic syphilis under these conditions: (*a*) when the patient is in good general condition from the standpoint of risk from toxic reactions; (*b*) when the patient is able to rest for at least twelve to twenty-four hours after injection to recover from minor reactions; (*c*) when the physician possesses experience and skill in its administration; also the time to devote to close attention to details and especially proper alkalinization of solutions and the slow injection of dilute solutions. It is not the arsenical of choice for the treatment of ambulatory patients who must attend to their daily work and duties without interruption; or for those who must be treated secretely and who therefore fear being incapacitated by toxic reactions; or for the busy physician who cannot spare the longer time required for its administration; or for individuals with difficult veins since injection by gravity is more difficult than the injection of concentrated solutions of neoarsphenamin or sulpharsphenamin by syringe methods; or for those patients who cannot afford the greater expense of an adequate course of arsphenamin injections since the physician must usually expect a larger fee because of the greater time and skill required for their administration.

But I believe that we have allowed the ease of administration of neoarsphenamin and sulpharsphenamin to overshadow the advantages of arsphenamin and I predict that we will come back again to the employment of arsphenamin more frequently than at this writing, when it is discovered, as I believe it will be, that the incidence of late tertiary lesions is greater after treatment with neoarsphenamin and sulpharsphenamin than after adequate courses of arsphenamin.

Advantages and Disadvantages of Silver Arsphenamin in the Treatment of Syphilis.—My experience with silver arsphenamin has been much less than with arsphenamin and has been confined entirely to American made products. I believe it is slightly more spirocheticidal than arsphenamin and it is said to be more efficacious than the latter in the treatment of neurosyphilis because of higher penetrability of the tissues of the central nervous system. Otherwise, in my opinion, it is not superior to arsphenamin in the treatment of either early or late syphilis. It is just as likely to produce toxic reactions and probably has the added danger of producing argyria when administered as frequently and in the dosage required for the adequate treatment of both early and chronic syphilis. It is easier to administer, however, than arsphenamin because the solutions do not require alkalinization. Small doses as 0.1 gm. dissolved in 20 c.c. of sterile water may be given by syringe injection, but the usual dose of 0.4 gm. requires gravity injection of dilute solutions. It is slightly more toxic for the lower animals than some brands of arsphenamin and in my experience, doses of 0.4 gm. are just as likely to produce toxic reactions in adult cases of syphilis as 0.4 gm. doses of arsphenamin. In my limited experience I have found it to have in general the same advantages and disadvantages of arsphenamin; I know of no special reasons for preferring it to the latter unless it be in the treatment of neurosyphilis, which opinion is based more upon a review of the literature than my personal experience with it in the treatment of these cases. Within recent years the use of silver arsphenamin has decreased; Michelson[1] has reviewed the literature up to 1921.

Advantages and Disadvantages of Neoarsphenamin in the Treatment of Syphilis.—Neoarsphenamin is undoubtedly the most widely used arsenical

employed to-day in the treatment of syphilis. The greater ease of administration, greater safety and greater freedom from untoward effects have quickly won for the compound greater favor with the majority of physicians than arsphenamin and other arsenical compounds. It was particularly popular during the World War in the armies of the United States, France and England, because of the ease of its administration and hundreds of physicians returning to civil practice have continued to use it in the treatment of syphilis. Its rapid solubility, ease of preparing solutions, the fact that concentrated solutions may be injected and low toxicity constitute its greatest advantages. For the lower animals it is at least two to four times less toxic than arsphenamin in alkaline solution and it is less likely to produce toxic reactions in human beings. These properties have encouraged the wide use of neoarsphenamin, have done more than any other single factor to foster the more thorough treatment of syphilis and has placed the modern treatment of the disease with the organic arsenicals within the reach of the majority of physicians instead of in the hands of relatively few specialists.

But neoarsphenamin is only about half as spirocheticidal as arsphenamin; in my experience, 0.9 gm. per 60 kilograms does not always or uniformly possess the same spirocheticidal activity as 0.6 gm. of disodium arsphenamin per 60 kilograms. As stated in Chapter XII, neoarsphenamin is much more likely to vary in trypanocidal and spirocheticidal activities than arsphenamin; this is not only true of neoarsphenamin prepared in different laboratories but to some extent with different batches prepared in the same laboratory. To-day there exists a remarkable variation in the parasiticidal effects of different brands of neoarsphenamin employed in the treatment of syphilis. I have repeatedly found samples on the market unable to cure syphilis of rabbits in doses as high as 0.045 gm. per kilogram whereas the average single curative dose should be 0.020 gm. per kilogram of weight for these animals. It is true that repeated doses may dissipate the obvious lesions of syphilis in human subjects and relieve some of the symptoms, but I doubt that the effects will be permanent and I believe that a high incidence of tertiary syphilis will be observed in the next ten to twenty years among syphilitics who believed themselves cured by series of injections of some brands of neoarsphenamin being employed to-day in the treatment of acute syphilis. Manufacturers of neoarsphenamin are laying entirely too much stress upon the flash solubility and low toxicity of their products; more attention should be given to their curative activity. In the competitive race for compounds of low toxicity, spirocheticidal activity has been overlooked and, I believe, that with some brands at least, technical procedures resulting in the lowering of toxicity for the rat have at the same time lowered toxicity for the spirochete of syphilis. I regard this situation as a serious one because I believe physicians and patients are not infrequently being deluded and I hope that the Hygienic Laboratory of the Public Health Service will establish a standard of parasiticidal activity for neoarsphenamin just as it has long since done for toxicity, with wonderful and praiseworthy results. Few realize better than I do what this entails in the way of work, expense and responsibility; indeed, tests with rabbit syphilis are out of the question on a broad scale because of the large number of animals required and since at least four months are required to obtain final results, but, as shown in Chapter X, trypanicidal tests are readily, rapidly and cheaply conducted and I am convinced that the trypanocidal activity of neoarsphenamin is a good and acceptable index of its spirocheticidal properties. I believe that manufacturers should be required to determine the trypanocidal activity of at least one out of every three to five batches of neoarsphenamin produced and that the Hygienic Laboratory

should check up the results at frequent intervals. I hope that physicians in general will take a greater interest in this subject and show the manufacturers that they are interested just as much in the question of curative activity as the physical qualities of the drug and its low toxicity.

As stated above, the trypanocidal and spirocheticidal properties of neoarsphenamin are apt to vary even among different lots dispensed by the same laboratory; for this reason I never treat a patient with the same lot number, but if 12 to 15 injections are to be given as a course, I endeavor to make up the series with at least two or three different lots of the compound. I believe this is a practical way for overcoming in part at least, the regrettable error of treating with a single batch of the drug below the average in curative activity.

Furthermore, as described in Chapter XII, neoarsphenamin is likely to deteriorate in the ampules and these changes affect not only the physical properties and toxicity but also to some extent the parasiticidal properties. Naturally if the product has oxidized slightly one would expect an increase of toxicity and an increase of parasiticidal activity, but I have found some lots of orange or brown color with reduced trypanocidal activity due, presumably, to far advanced oxidation. It is advisable not to use neoarsphenamin more than a year old even though it appears acceptable from the standpoint of physical properties; pharmacists sometimes overstock and dispense to physicians neoarsphenamin several years old and this practice is to be condemned and carefully avoided.

Neoarsphenamin, therefore, possesses the great practical advantages of ease of administration and safety; it is the remedy of choice to-day for the office treatment of ambulatory patients requiring arsenical therapy who cannot afford to be incapacitated by even minor reactions. The ease of administration also greatly reduces the expense; at least it should do so, and thereby enable patients of average or reduced financial status to undergo more thorough treatment than in the pre-neoarsphenamin days. But it must always be remembered that the drug is only about one-half as curative as arsphenamin at best, that if poor products are used treatment will be insufficient with the danger of producing arsenic fastness of surviving spirochetes and that more injections and larger doses are thereby required; also that the compound varies in curative activity and is more likely to deteriorate and therefore requires more vigilance on the part of the physician. It is possible to secure neoarsphenamin in which the dose of 0.9 gm. is practically equivalent in curative activity to 0.6 gm. of arsphenamin and with such I believe that neoarsphenamin is the best and most satisfactory arsenical available to-day for the routine treatment of syphilis even though arsphenamin, silver arsphenamin or sulpharsphenamin may be indicated in special cases.

Advantages and Disadvantages of Neosilver-arsphenamin in the Treatment of Syphilis.—Neosilver-salvarsan has been used in Germany during the past three years and a number of reports have been rendered by Weber and Hillenberg,[2] Dub,[3] Weber and Hillenberg,[4] Duhot,[5] Krebs,[6] Ziegler,[7] Steuhmer,[8] Bruhns and Bluemener,[9] Liebner and Rado,[10] Dreyfus,[11] Galewasky,[12] and others, showing in a general manner that it is somewhat superior to neoarsphenamin in that the solutions do not oxidize as rapidly and are more stable, that toxic reactions are less frequent and that spirocheticidal effects are somewhat greater. To the best of my knowledge the compound has not been employed in America and I have had no experience with it at all either clinically or in rabbit syphilis. The data and arguments in favor of adopting it in preference to neoarsphenamin and sulpharsphenamin, however,

would have to be quite conclusive to convince me to employ the compound, because I believe extravagant claims have been made for silver arsphenamin and I greatly dislike giving a series of intravenous injections of silver even though the danger of producing argyria may be more theoretical than established fact.

Advantages and Disadvantages of Sulfarsenol or Sulpharsphenamin in the Treatment of Syphilis.—*Sulfarsenol*, a French arsenical composed of arsenobenzol with formol and sodium acid sulphite, but the method of preparation of which is kept a trade secret, has been employed mostly in Europe in the treatment of syphilis since 1920; *sulpharsphenamin*, a sulphurous acid ester of arsphenamin made from arsphenamin, formaldehyd, and sodium bisulphite, was prepared by Voegtlin, Johnson and Dyer[13] in 1922, and is believed to be closely similar to sulfarsenol in preparation and identical in properties; it is being widely employed at present in America.

The latter preparation is quite similar to neoarsphenamin, differing by the presence of one additional atom of oxygen; it contains from 22 to 24 per cent. of arsenic, is easier to manufacture and possesses greater stability in both dry form and in water solution. Both sulfarsenol and sulpharsphenamin possess the same ease of administration as neoarsphenamin, since they are quickly soluble, do not require alkalinization and may be administered in concentrated solutions.

In my experience, sulpharsphenamin is slightly less spirocheticidal in rabbit syphilis than the best brands of neoarsphenamin when both are given by intravenous or both by intramuscular injection in the same doses per body weight; I have had much less experience with sulfarsenol, but the lots which I have tested have been uniformly less trypanocidal and spirocheticidal than sulpharsphenamin and I have no hesitation whatsoever in expressing a preference for the latter even though they are supposed to be practically identical in chemical composition.

As stated in Chapter XXIX, arsphenamin, neoarsphenamin and sulpharsphenamin are distinctly more trypanocidal and spirocheticidal in experimental infections by intramuscular than by intravenous injection, but the first two produce so much local reaction in doses of 0.2 gm. or more that they cannot be given by subcutaneous or intravenous injection whereas sulpharsphenamin may be administered by this route if not more than 0.6 gm. is deposited in any one area. Herein lies the chief advantage of sulpharsphenamin. When administered to syphilitic rabbits and to human beings with chancres and secondaries by *intravenous injection*, sulpharsphenamin, in my experience, has been slightly less curative than the best brands of neoarsphenamin and only about one-half as curative as arsphenamin; but sulpharsphenamin in dose of 0.6 gm. by *intramuscular or subcutaneous injection* is usually more spirocheticidal than 0.6 gm. of neoarsphenamin by intravenous injection and almost equal to arsphenamin by intravenous injection, although the average results have shown that the latter is slightly but distinctly more spirocheticidal.

In other words, there is no use in my opinion comparing sulpharsphenamin with neoarsphenamin by intravenous injection; I am convinced that the best brands of the latter are superior to the best brands of the former in curative activity by this route of administration, although the difference is slight and much better elicited by careful animal experiments than by clinical studies and observations. Likewise, there is no use in comparing sulpharsphenamin with neoarsphenamin by intramuscular injection, insofar as curative activity is concerned; on the basis of trypanocidal tests with rats and spirocheticidal tests with rabbit syphilis, the latter is more active. But

neoarsphenamin cannot be given adult cases of syphilis by intramuscular or subcutaneous injection in sufficiently large doses, whereas sulpharsphenamin may be given by these routes and undoubtedly 0.4 gm. of sulpharsphenamin by intramuscular injection is much more spirocheticidal than 0.4 gm. of neoarsphenamin in intravenous injection. But how does a dose of 0.4 gm. of sulpharsphenamin per 60 kilograms of weight by intramuscular injection compare in parasiticidal activity with 0.9 gm. of neoarsphenamin per 60 kilograms by intravenous injection? In my experience, the brand of neoarsphenamin which I have used has proved equal to or slightly better than the two brands of sulpharsphenamin employed and decidedly better than the one brand of sulfarsenol. I am convinced that sulpharsphenamin and sulfarsenol by intravenous injection are inferior in spirocheticidal activity to the brand of neoarsphenamin which I have used and that the latter is to be preferred when intravenous injections are to be given in the treatment of *acute early* syphilis. But in the treatment of *chronic* syphilis and especially of neurosyphilis, it would appear that intramuscular injections of sulpharsphenamin are superior to intravenous injections of neoarsphenamin; I base this statement almost entirely upon the work of Voegtlin, Smith, Dyer and Thompson,[14] showing that it has superior penetrative properties for the brains and spinal fluids of rabbits and more particularly upon the recent clinical report of Stokes and Behn,[15] rather than upon my own limited clinical experience. The latter report is the best so far appearing in the American literature, although numerous reports on sulfarsenol have appeared especially in France by Levy-Bing, Lehnhoff-Wyld and Gerbay,[16] Bernard,[17] Yernaux and Bernard,[18] Emery and Morin,[19] Hewins and Acre,[20] Doble,[21] Irgang,[22] Asch,[23] Boone and Weech,[24] Crawford and Fleming,[25] Durveux,[26] Papegaay and Rinsema,[27] Belding,[28] and others. It is not profitable for me here to review these reports in detail; some are favorable and some are unfavorable, but the general tenor has been to emphasize the fact that sulfarsenol or sulpharsphenamin may be given by either subcutaneous or intramuscular injection, that by these routes it has at least the curative activity of neoarsphenamin by intravenous injection and that toxic reactions with the exception of dermatitis, are fewer and less serious. Stokes and Behn, injecting 0.4 gm. in 1.2 to 1.5 c.c. of distilled water at 3 to 5 day intervals, have found one brand of sulpharsphenamin superior to neoarsphenamin in acute early as well as chronic, cardiovascular, neural and Wassermann-fast cases and they state that the drug by this route of administration has proved to be quite as effective therapeutically as arsphenamin and neoarsphenamin from the standpoint of spirillicidal action and of the effect on the blood Wassermann and spinal fluid reactions, on the healing of lesions, and on the general well-being of the patient. They believe they saw distinct superiority, in the treatment of neurosyphilis over the older drugs, and distinct evidence of superiority over neoarsphenamin intravenously in all aspects of syphilis. They have likewise given the most favorable report on the degree of local reactions of pain and tenderness in the muscles; they divided the dose 0.4 gm. between the two buttocks and gave the injections epifascially (intermuscularly) instead of intramuscularly. Most likely these technical factors account for the fact that, in 83 per cent. of their 1,360 injections, there was no local discomfort whatsoever. I thoroughly agree with them that it is well not to inject more than 0.2 to 0.4 gm. in one area; a few of my patients have complained bitterly and a few have absolutely refused injections of 0.4 to 0.6 gm. dissolved in 2 to 4 c.c. of sterile water in one area, although the sulpharsphenamin being dispensed at the present time as much less painful than formerly.

Belding has called particular attention to the high incidence (about 16 per cent.) of skin reactions following injections of sulpharsphenamin. I have observed the same in about 5 per cent. of injections and believe that intravenous injections are more likely to produce dermal accidents than intramuscular injections. It is noteworthy that Stokes and Behn observed only 3 per cent. of toxic reactions, two patients developing exfoliative dermatitis. If I understand their report correctly, the incidence of dermatitis was 1 to about 700 injections as compared with 1 to 4,300 injections of arsphenamin. This incidence is certainly much lower than my own, although I have included such mild reactions as localized urticaria and erythema, the development of which always induces me to stop the administration at once in order to avoid the possibility of producing the dreaded severe forms of dermatitis. It is interesting to note that Stokes and Behn gave injections of sulpharsphenamin without mishap to five individuals who had developed cutaneous reactions from arsphenamin and neoarsphenamin.

To sum up, the chief advantages of sulpharsphenamin are ease of administration, that subcutaneous or intramuscular injections may be given and that, by the latter route, the compound possesses the same or better specific spirocheticidal effects than neoarsphenamin by intravenous injection. For my own part, I still prefer intravenous injections of the neoarsphenamin I have been using for years for the treatment of acute early syphilis; for the treatment of chronic syphilis of adults and especially neurosyphilis and congenital syphilis of children however, I believe that sulpharsphenamin by intramuscular injection is worthy of trial and as manufactured at present skin reactions are much less frequent than observed one or two years ago when this product was first placed upon the market. As stated by Boone and Welch[28a] sulpharsphenamin by intramuscular injection is particularly well adapted for the treatment of congenital syphilis and Fergusson[28b] and others have found it well adapted for the treatment of syphilis afloat on war vessels. More recently Fordyce, Rosen and Myers[28c] have reviewed the literature on sulpharsphenamin along with their own laboratory and clinical investigations and state that the compound must be used with caution from the standpoint of reactions and purpura, that it is two and one-half times less active than neoarsphenamin therapeutically, that it has not shown any special affinity for the central nervous system and that clinically, no superior results were noticeable.

Advantages and Disadvantages of Sodium Arsphenamin, Sulphoxylate Arsphenamin and Galyl in the Treatment of Syphilis.—Sodium arsphenamin was produced in Germany in the early part of 1915, and has enjoyed a brief popularity as a substitute for arsphenamin, inasmuch as the solutions in water do not require alkalinization and concentrated solutions may be injected in exactly the same manner as neoarsphenamin.

In America, the drug has been used to some extent but has been gradually superseded by neoarsphenamin. The literature up to 1921 is mostly in German and has been well reviewed by Michelson and Siperstein,[29] so that I shall omit here the references. As stated in Chapter XII, I have found this compound slightly less trypanocidal than neoarsphenamin although its spirocheticidal effects in rabbit syphilis were about equal to neoarsphenamin insofar as could be judged in a few experiments. From the standpoint of ease of administration, toxicity and curative activity it is about on par with neoarsphenamin, although it is more toxic for rats and rabbits and, therefore, possesses a lower chemotherapeutic index than neoarsphenamin; it is certainly less curative than arsphenamin and produces about as much local reaction as neoarsphenamin so that it cannot be given intramuscularly. On the

whole, I believe the compound is slightly less efficient than the best brands of neoarsphenamin and, as stated above, it would appear that the latter is preferred by most physicians in the treatment of syphilis.

Sulphoxylate arsphenamin is a different preparation of secret manufacture. It is marketed as a fluid but has not been extensively used in Germany and not at all in America. Kumer[30] thought it had as much spirocheticidal activity in the treatment of rabbit syphilis as neoarsphenamin, but these observations were not confirmed in human cases of syphilis. To the best of my knowledge the compound is no longer employed for the treatment of syphilis.

Galyl or tetraoxydiphosaminodiarsenobenzene is also a secret preparation first prepared by Mouneyrat of Paris and named after Galan, who first noted the properties of arsenic. According to an analysis by the A. M. A. Chemical Laboratory,[31] the new "galyl" decomposes in water into sodium phosphate and sodium arsphenamin or else contains sodium arsphenamin and sodium phosphate, its injection apparently amounting to the administration of a mixture of phospharsphenamin (if any is present), sodium arsphenamin, sodium phosphate, sodium sulphite and sugar. During the World War the preparation was apparently widely used in Europe, since it was dispensed with sufficient sodium carbonate to enable one to dissolve it in water and administer by intravenous injection with the same ease as sodium arsphenamin or neoarsphenamin. However, but few reports on its use in syphilis have appeared among which may be mentioned those of Beurmann, Mouneyrat and Tanon[32] and Troisfontaines[33] in France; Foerster,[34] Spense[35] and Dudley[36] in England, and Bruner[37] and Thompson[38] in America.

In my laboratory, experiments have shown the compound about as toxic as arsphenamin for rabbits and rats with the trypanocidal and spirocheticidal activity of sodium arsphenamin; its chemotherapeutic index, therefore, is low. I have had no clinical experience with the compound but from laboratory experience and a review of the literature I regard it inferior to sodium arsphenamin and much inferior to the best brands of neoarsphenamin.

Advantages and Disadvantages of Pentavalent Arsenical Compounds as Sodium Cacodylate, Tryparsamid, Etc., in the Treatment of Syphilis.—The first organic compounds of arsenic to be used in the treatment of syphilis were such pentavalent compounds as *atoxyl* and *arsenophenolglycin*. Insofar as the treatment of syphilis is concerned, they are now only of historical interest since the doses required for specific spirocheticidal effects are too toxic, sometimes producing amblyopia in the case of atoxyl and skin reactions in the case of arsenophenylglycin. *Sodium cacodylate* also enjoyed a brief popularity for a time on the basis of causing the disappearance of chancres and secondaries, which is an example of how easy it is to foster new compounds upon the medical profession because of the comparative ease with which these lesions may be dissipated, as shown by the report of Marechal[39] on the use of two methylarsinates, sodium cacodylate and "arrhenal;" but since the unfavorable reports of Cole,[40] Nichols[41] and others with "mon arsone" these compounds have been discarded in the treatment of syphilis.

Pomaret and other French investigators, however, continue to enlist interest in the pentavalent arsenicals and especialy in the sodium salt of aminophenylarsenic acid ("eparseno" or "132"), a derivative of Ehrlich's "592," the sodium salt of oxyamino-phenylarsenic acid ("189") and other compounds of the same general class. Pomaret and Bloch,[42] Lepinay[43] Sherwood-Dunn[44] and others, for example, have warmly endorsed sodium aminophenol in doses of 0.12 to 0.36 gm. by intramuscular injection and state that the local pain is not greater than produced by mercury while the curative effects are equal to neoarsphenamin. They claim that the toxic effects are largely due to impurities removable by closer attention to technical details in manufacture; Fournier, Guénot and Schwartz[45] and others have made similar claims for the sodium salt of oxyaminophenylarsenic acid and Levaditi, Navarro-Martin, Fournier, Guénot and Schwartz[46] and Laurent[47] for the sodium salt of acetyloxyaminophenylarsenic acid ("stovarsol," "acetylarsan" or "190") by oral administration. I have given in previous

chapters the results of trypanocidal, spirocheticidal and toxicity studies in the lower animals; I have had no clinical experience with the compounds, but feel convinced that they are inferior to sulpharsphenamin in the treatment of syphilis by intramuscular injection, are more toxic and have no superior claims warranting their choice for the treatment of syphilis although they and the pentavalent compounds of arsenic in general, may be more useful in the treatment of trypanosomiasis. The only pentavalent arsenical compounds worthy of special consideration are *tryparsamid* and *stovarsol*, the former by intravenous injection in the treatment of neurosyphilis according to the reports of Lorenz, Loevenhart, Bleckwenn and Hodges,[48] Moore, Robinson and Keidel[49], Lorenz, Loevenhart, Reitz and Eck,[50] Hindman[51] and others which I will review with more detail in Chapter LXXVI, devoted to a consideration of the treatment of neurosyphilis. This compound is apparently able to penetrate the tissues of the brain and cord and, according to Lorenz and his colleagues, is especially useful in conjunction with intramuscular injections of mercury salicylate in the treatment of early paresis, meningovascular syphilis, and, to a lesser extent, in taboparesis, tabes and advanced paresis. The drug is of no particular use, however, in the treatment of acute, early syphilis or other forms of chronic syphilis and its application is limited by the fact that it produces visual disturbances through involvement of the optic nerves in 7 to 23 per cent. of cases, as is so likely to be the case with the pentavalent arsenicals in general in contradistinction to the trivalent arsenicals like arsphenamin, neoarsphenamin and sulpharsphenamin which do not produce these toxic amblyopias.

Stovarsol is given by mouth in dose of 4 tablets (0.25 gm. each) usually with a little water before breakfast each day for 5 to 7 days; after a rest period of a week the course is repeated so that in a month as much as 12 to 16 grams have been given by Levaditi, Fournier and other French clinicians. This remedy however, is not free from unpleasant or even injurious toxic effects; moreover, its effect upon the various manifestations of syphilis, and especially on the Wassermann reactions, seems to be less than that of arsphenamin and its congeners. It is probable however, that the compound may prove of service when intravenous or intramuscular injections cannot be given and under these circumstances is worthy of trial.

Advantages and Disadvantages of Mercury in the Treatment of Syphilis.—The position of mercury in the treatment of syphilis is still upon the firm foundation of clinical experience. Threatened with dismissal by the discovery of salvarsan and ignored for a few years, it has since become again firmly established in the opinion of most syphilologists as indispensable and especially in the treatment of chronic syphilis. It is true that during the past ten years not a few reports have appeared stating that syphilis may be treated and cured by arsphenamin or its substitutes alone, but for every one physician of this opinion there are at least one hundred who are convinced that mercury should be given at some stage in the treatment of syphilis in all of its stages. Arsphenamin and its substitutes have not displaced mercury; they have simply proven additional and powerful weapons for treating syphilis and especially in its early stages. At the present time the position of mercury is again threatened by the advent of bismuth compounds, but in my opinion the newcomers will not displace mercury completely, although they are proving to be additional weapons at our command for combating syphilis and especially in the chronic stages.

Mercury is a spirocheticide, but is likewise so toxic that sufficiently large doses cannot be given at one time to produce the spectacular results of arsphenamin and its substitutes. For example, the largest dose of bichlorid

that can be given a syphilitic rabbit by intravenous injection is about 0.003 gm. per kilogram of weight, but I have repeatedly found that spirochetes disappear from scrotal and testicular lesions with about the same rapidity as when 0.003 gm. per kilogram of disodium arsphenamin is given intravenously. If we could safely give repeated injections of a rapidly dissociable soluble salt of mercury, like the bichlorid, intravenously in a dose even one-half as large as that of arsphenamin, the results would be just as pronounced as with the latter; but this, of course, is not possible on account of the high toxicity of the compound. It is true that the mercurial compound flumerin may be given in a dose as high as 0.005 gm. per kilo, which for an adult of average size corresponds to 0.3 gm. or one-half the usual dose of arsphenamin; but the results are not as good as a similar dose of the latter, because the mercury of this compound is not ionized as rapidly as in the case of inorganic salts like bichlorid. The high toxicity of mercury for the body constitutes, therefore, its great disadvantage. It requires that mercury be given in small repeated doses over a long period of time; it does not permit the administration of large sterilizing doses over a short time as is so easily possible with arsphenamin and its substitutes.

As a result, mercury cannot be given in sufficiently large doses to produce rapid spirillicidal effects. It is true that spirochetes disappear from lesions with healing of the latter, but the effects are not rapid enough in the majority of cases to prevent some of the organisms escaping destruction with subsequent localization in the internal organs and the production of chronic lesions. For this reason mercury is not the remedy of choice to-day for the treatment of acute early syphilis when rapid spirillicidal effects are demanded; the trivalent arsenicals arsphenamin and its substitutes are the remedies of choice at this stage with bismuth second and mercury third.

But mercury is of value in tertiary syphilis and why should this be so if it is so inferior in spirillicidal effects in the doses we may safely administer? An exact answer cannot be given except to state that clinical experience supports the statement. It is my belief that the reason is that mercury tends to accumulate in syphilitic tissues and exudates just as the iodids do in any syphilitic or non-syphilitic exudate; that because of this affinity of mercury for syphilitic tissues, it gradually reaches a concentration of spirocheticidal proportions without at the same time reaching toxic concentrations in the kidneys, liver and other important organs sufficient to interfere with their functions or with the elimination of mercury. To the best of my knowledge, this stored or accumulated mercury is still ionizable and therapeutically active, even though organic combinations may be produced with the cellular and humoral proteins. Arsphenamin and its substitutes, however, do not have this tendency for accumulation in syphilitic exudates, because arsenic does not have the same affinity although it may accumulate in the liver, bone marrow and other organs; or, if it does accumulate, it is rendered therapeutically inactive by processes of oxidation or reduction depending upon the nature of the compound with the same final result in either case, namely, therapeutically inactive pentavalent arsenic. In other words, the period of therapeutic usefulness of arsphenamin and its substitutes in the body is relatively brief; a few hours may be required to oxidize them into the spirocheticidally active oxids but unfortunately the process does not stop here: oxidation continues with the formation of compounds less active or inert. Arsphenamin, therefore, is like a "broom that sweeps," to quote Stokes, whereas faithful old mercury takes up residence in syphilitic foci in a fighting condition and is the weapon that "crushes" the spirochetes. Anything like a fibrous tissue barrier may prevent arsphenamin gaining entrance during its brief and fleeting

visit to the strongholds of the enemy; mercury is slower, knocks more persistently, is in no hurry to leave and finally gains entrance with death to spirochetes.

Others are of the opinion that mercury cures by stimulating immunity processes. No one expressing this view has to my knowledge substantiated the statement and as discussed at length in Chapter XXIV, I have not for myself been able to elicit evidence in favor of it. If mercury does act in this way, it probably does so by releasing antigenic substances from spirochetes which in turn engender the production of antibodies. Possibly our knowledge and technic are not sufficient at the present time for detecting the nature and degree of immunological changes, but for my own part I believe that mercury influences syphilis by the direct spirocheticidal effects of ionized mercury, as described above and in Chapter XXIV.

Naturally a very large number of different compounds and preparations of mercury have been advocated and employed in the treatment of syphilis; within the last two or three years various new organic compounds with dyes have commanded special attention like the mercury phthaleins, mercury-anthrachinons and mercury-azodyes studied by Hahn and Kostenbader;[51a] flumerin (the disodium salt of hydroxymercuri-fluorescein) described by White and his colleagues;[51b] a similar fluorescin-mercury compound described by Stuemer[51c] under the name of "Tachysan S;" a colloidal preparation of mercury amalgamated with gold described by Snodgrass[51d] and designated as "contraluesin," etc., but definite opinions of the value of some of them in the treatment of syphilis cannot be expressed at present.

The high toxicity of mercury is not to be mentioned as a disadvantage, except that it interferes with the administration of sufficiently large amounts at one time to duplicate the rapid mass spirocheticidal effects of arsphenamin; it is not, therefore, the remedy of choice in the treatment of acute early syphilis (primary and secondary stages). Its toxic effects are well understood and easily prevented, although some individuals will not tolerate it at all in therapeutic doses, but this is true of almost every other therapeutically worth while medicament for syphilis and other diseases as well. For the treatment of chronic syphilis in which mercury is still to be regarded as one of our most valuable remedies, it is usually possible to administer the compounds over long periods of time without injury to the patient.

Advantages and Disadvantages of Bismuth in the Treatment of Syphilis.—Compounds of bismuth are the latest additions to the family of remedies for the treatment of syphilis, being brought prominently before the profession in 1921–1922 by the laboratory investigations of Sazerac and Levaditi[52] and the clinical studies of Fournier and Guenot.[53] I have reviewed in Chapters XV and XXV the results of toxicity, trypanocidal and spirocheticidal activities, giving the main references to the literature. Since then a very large number of publications have appeared in France, Italy, Germany and Switzerland, with fewer in England and America, this large literature having been recently summarized by Levaditi[54] showing the curative activities of bismuth and especially the tartrobismuthates, in primary and secondary syphilis as well as in chronic syphilis and particularly neurosyphilis; it is not necessary, therefore, for me here to repeat this review which is very complete up to 1924. Since then, the reports by Klauder,[55] Hopkins,[56] McCafferty[57] and Shivers[58] have been noteworthy contributions from America.

My experience with bismuth has been confined entirely to potassium tartrobismuthate, sodium tartrobismuthate and the quartenary salt sodium and potassium tartrobismuthate. As would be expected a large number of newer compounds have already appeared, each with its usual claims of superiority. One of the latest of these, which I believe is worthy of special mention, is a bismuth nuclein compound reported upon by Fournier, Guénot,

Schwartz and Yovanovitch[59] because the combination with protein suggests that its administration may be accompanied by the spirillicidal effects of bismuth plus the non-specific protein reaction of the nuclein.

Weight for weight bismuth in the form of the sodium and potassium tartrobismuthate possesses in my experience about one-fourth to one-fifth the spirocheticidal activity of arsphenamin for rabbit syphilis, the single curative doses being approximately 0.050 gm. for bismuth and 0.012 gm. for arsphenamin (disodium salt) per kilogram of weight; contrary to popular opinion, it possesses only one-tenth the spirocheticidal activity of mercury. It is, however, twenty to forty times less toxic than mercury so that its chemotherapeutic index is at least five times higher, but weight for weight, bismuth is less spirocheticidal than either arsphenamin or mercury. However, owing to its lower toxicity we can administer much larger doses in the treatment of syphilis and this accounts for the impression that it is actually more spirocheticidal. For example, we may ordinarily give 0.2 gm. of the tartrobismuthates every 5 days for 15 doses or a total of 3.0 gm. before siderosis and other toxic effects appear; this corresponds to between 3 and 4 grains per dose or a total of 45 to 50 grains in a matter of 75 days, which is at least four to five times more than we give of mercury, since the dose is usually about 1 grain per week. In other words, mercury is about as toxic for body cells as it is for *Sp. pallida;* hence its chemotherapeutic index is only about 1, which constitutes its chief disadvantage. Bismuth, on the other hand, is about five times more toxic for *Sp. pallida* than for body cells; hence, its chemotherapeutic index is about 5 which constitutes its chief superiority over mercury. Arsphenamin is about 10 times more toxic for *Sp. pallida* than for body cells; hence its chemotherapeutic index is about 10. The best brands of neoarsphenamin and sulpharsphenamin are fourteen to eighteen times less toxic for body cells; hence their chemotherapeutic indices are 14 to 18. This tells the story of preference of compounds for the treatment of acute early syphilis when "sweeping" effects alone may suffice; undoubtedly the preference should be first for arsphenamin, neoarsphenamin or sulpharsphenamin; second, for bismuth and lastly, for mercury.

In the treatment of acute syphilis, either in the primary or secondary stages or acute progressive exacerbations of chronic lesions, bismuth would appear to be the medicament of choice if arsphenamin and its substitutes cannot be given on account of hypersensitiveness or other cause; this is because we may give safely larger doses of bismuth by injection than we may of mercury and thereby secure the next best rapid, mass spirocheticidal effects.

But what is the status of bismuth in the treatment of chronic syphilis? I doubt if this question can be fully answered at the present time; I know that my own mind is far from being clear on this point. In view of the fact that the chemotherapeutic index of bismuth is about five times higher than that of mercury in the way of single curative doses for rabbit syphilis, it would appear that the answer is apparent, but the treatment of chronic human syphilis presents problems that cannot be answered by experiments with either acute or chronic syphilis of the rabbit or monkey. I am of the opinion that bismuth exerts its curative effects by the spirocheticidal activity of the bismuth ion just as, I believe, mercury is curative by the spirocheticidal activity of the mercury ion. But we do not yet know if bismuth has any special affinity for syphilitic tissues as I believe mercury does; nor whether it is better able to penetrate fibrous tissue barriers and reach spirochetes. It is true, however, that clinical reports indicate an undoubted curative influence of bismuth upon tertiary lesions and especially in some forms of neuro-

syphilis as shown by Shivers, but I doubt the wisdom of substituting bismuth for mercury entirely in the treatment of chronic syphilis, even though one is tempted to do so since bismuth is so much less toxic than the latter. Furthermore, the oil suspensions of potassium tartrobismuthate or of sodium and potassium tartrobismuthate in dose of 0.1 to 0.2 gm. (2 to 3 grains) by intramuscular injection are so much less painful and so seldom produce tender nodosities as compared with 1 grain doses of any of the insoluble mercurials in oil, that patients very much prefer them and, indeed, a few refuse the mercury injections after once experiencing the greater comfort of the former. A review of the literature along with my own experience with intramuscular injections of oily suspensions of potassium tartrobismuthate and of sodium and potassium tartrobismuthate in doses of 0.1 to 0.2 gm. every five to seven days have lead me to formulate the following conclusions on the curative activity of bismuth in syphilis:

1. Spirochetes sometimes disappear from chancres within forty-eight hours after the first injection and almost always after 1 to 3 injections; chancres have usually healed within seven to twenty-one days.

2. Mucous patches in the mouth usually disappear very rapidly but the cutaneous secondary lesions more slowly and sometimes are so refractory that arsphenamin or neoarsphenamin must be administered.

3. The tartrobismuthates usually quickly heal open infectious lesions, but in my experience the action is not usually as prompt as follows the intravenous administration of 0.6 gm. of arsphenamin or 0.9 gm. of neoarsphenamin.

4. Tertiary lesions usually respond quite promptly and especially those of the skin and periosteum.

5. In some cases of neurosyphilis and especially cases of chronic syphilitic meningitis with persistent headache, the results are brilliant and frequently superior to those observed with mercury and the organic arsenical compounds. In tabes dorsalis the pains are usually promptly relieved by a few injections.

6. In congenital syphilis the lesions usually respond as promptly as under treatment with mercury or neoarsphenamin; cases of syphilitic epilepsy have been especially benefited.

7. Bismuth has proved especially useful in the treatment of syphilitic patients who are hypersensitive to arsphenamin, neoarsphenamin or mercury.

8. Bismuth is sometimes curative in cases of syphilis resistant to mercury and the organic arsenical compounds; in Wassermann-fast cases a form of combination theory with bismuth and an arsenical frequently yields better results than treatment alone with an arsenical or mercury compound.

9. Bismuth does not produce Herxheimer reactions as frequently as the organic arsenical compounds and is very useful for starting the treatment of chronic active or latent syphilis when "neurorecidives" and such exacerbations of syphilitic processes are feared and to be avoided.

10. Bismuth influences the Wassermann reaction more promptly than mercury but less promptly than neoarsphenamin and much less promptly than arsphenamin. In primary syphilis if treatment with bismuth is begun before the Wassermann test with serum yields a positive reaction, the reaction usually remains persistently negative indicating spirocheticidal activity.

To sum up, I believe that bismuth has established for itself a permanent place in the treatment of syphilis by reason of its low toxicity for the body associated with marked spirocheticidal properties; that it is especially indicated in the treatment of acute syphilis when arsphenamin and its substitutes cannot be given; that it is of value in the treatment of chronic syphilis and

especially of the central nervous system; that it is usually well borne when mercury is not and, therefore, serves a valuable substitute for mercury in chronic syphilis when the latter cannot be given in adequate dosage. Otherwise I am unwilling to use it as a substitute for either arsphenamin and allied compounds or mercury, until we know more of its final results. I believe, however, that it is worthy of use in the treatment of syphilis in all of its stages along with the organic arsenicals and mercury, as a form of combination therapy, which is the key note of success in the treatment of either acute or chronic syphilis.

Advantages and Disadvantages of the Iodids in the Treatment of Syphilis.—The great advantage of the iodids in the treatment of syphilis is their resolving influence upon organized syphilitic exudates or gummas. This is ordinarily so pronounced and especially on gummas of the skeletal system as to give the impression that the iodids are specific spirocheticides. On the contrary, they are not specific for syphilis since similar effects are sometimes observed in other infectious granulomas like sporotrichosis, blastomycosis and actinomycosis; neither are they strongly spirocheticidal since the iodids in the usual small doses are almost without destructive effects upon *Sp. pallida*. But they promote autolysis of gummas and less organized exudates and cause them to disappear more rapidly than in treatment alone with arsphenamin or mercury.

Some physicians still persist in treating cases of acute early syphilis by the iodids alone; some clinical improvement may follow, although Fournier has shown that the disease progresses through its usual stages with the production of tertiary lesions. There may be an excuse for treating some cases of tertiary syphilis with iodids alone because of their resolving action upon gummas, but there is absolutely no reason at all for using them alone in the treatment of acute, early syphilis; to do so constitutes a serious error.

When the treatment of syphilis is begun within eight weeks after the appearance of the chancre, there are no special indications for the administration of the iodids because there is no special work for them to do, since the exudates about the spirochetes are still cellular with no fibrotic changes in the blood-vessels; spirocheticides, as arsphenamin and its substitutes, mercury or bismuth may readily penetrate the exudates and reach the spirochetes. After the first few months of treatment, however, it is well to introduce the iodids for the purpose of aiding in reaching and destroying those spirochetes which have escaped destruction and brought about the laying down of fibrous tissue in the mesial coats of blood-vessels, perivascular spaces and inflammatory exudates.

When the treatment of syphilis is begun or resumed in the chronic stages of the disease, the administration of the iodids should constitute a very important part of treatment at the outset; I believe their administration should start with the first dose of arsphenamin, mercury or bismuth and persist throughout the course, being omitted during the rest periods since intermittent iodid treatment is better than continuous or unremitting medication. Even though there are no discernible gummas, yet the iodids doubtless aid in the removal of exudates of microscopic size in the different organs and more particularly in and about the walls of capillaries, thereby facilitating the access of spirocheticides, as arsphenamin or its substitutes, mercury or bismuth.

The iodids, therefore, have a place in the treatment of all cases of syphilis; in acute early syphilis during the first few months of intensive treatment with arsphenamin or its substitutes they may be omitted, since they serve no particular purpose at this time and may add to difficulties by producing toxic

reactions; but after this period, their administration is indicated and especially when gummatous deposits are seen or their presence in internal organs suspected by reason of symptoms and dysfunction.

The only disadvantages attending the administration of iodids are such toxic effects as the coryza, acne, etc. As stated in Chapter LVII, the iodids are not dangerous in the sense of producing tissue changes as the arsenicals, mercurials and bismuth compounds may produce upon prolonged administration. In this respect the iodids are eminently safe remedies. But it is to be remembered that the effects of iodids upon promoting the resolution of inflammatory exudates is not confined to syphilis and that their administration to tuberculous individuals may result in the breaking down of tuberculous lesions with dissemination of the bacilli; therefore, they must be used with great caution, if at all, when advanced tuberculosis is known to be present.

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CHAPTER LXVIII

NON-SPECIFIC PROTEIN THERAPY OF SYPHILIS

Non-specific Drugs in the Treatment of Syphilis.—In view of the high toxicity of mercury and the severe toxic effects sometimes produced by the administration of excessive amounts, it is but natural that many vegetable drugs have been advocated from time to time in the past as substitutes for the treatment of syphilis. But none have gained a place in the modern treatment of this disease although it is possible that intramuscular and intravenous injections of various vegetable proteins may prove of service, as would appear to be the case with proteins of animal origin, like intramuscular injections of sterile milk.

Probably the best known and most valuable of the non-specific drugs in the treatment of syphilis are the iodids. As ordinarily administered, these compounds are not specific in the sense of being sufficiently spirocheticidal to cure syphilis by killing spirochetes, as has been discussed in Chapter XXVI. Their feeble spirochetal activity has been abundantly shown in experimental syphilis and is confirmed clinically by the feeble curative activity of the iodids in the treatment of syphilis in its primary and secondary stages, as well as by the very high incidence of tertiary lesions in cases of syphilis treated exclusively with the iodids. But no one can deny their great value in the treatment of chronic syphilis by their influence upon the products of syphilitic infection, namely, in stimulating the autolysis of organized or fibrotic syphilitic exudates. The iodids, therefore, are not truly specific in syphilis; they are wonderfully curative non-specifics too frequently forgotten now-a-days in our concentration upon the specifics. They afford symptomatic relief when used alone; when administered along with specifics as the arsphenamin and mercurials, they aid in addition to bring about the destruction of spirochetes.

Non-Specific Protein Agents in the Treatment of Syphilis.—But non-specific therapy in syphilis refers more especially to the administration of agents capable of producing fever, leukocytic and enzymic changes, increase of capillary permeability, etc., and within recent years several publications

have shown that various non-specific agents may favorably influence the treatment of syphilis. I refer especially to *intramuscular injections of milk and milk preparations, subcutaneous injections of tuberculin, subcutaneous injections of luetin, including, possibly, the inoculation of paretics with malaria and relapsing fever*, etc. Schacherl[1] has reported that cases of syphilitic neuro-arthropathies and various types of neurosyphilis appeared to be benefited more by combined treatment with tuberculin and mercury than by mercury alone; the Wassermann reaction appeared to be much more rapidly changed and the mercury (salicylate) appeared to be absorbed more rapidly. Schreiner,[2] Uddgren,[3] and others have noticed that the Wassermann reaction became negative much sooner in cases treated with injections of milk and peptone along with specifics than by specifics alone and I have already referred to these observations in preceding chapters. Kryle[4] has reported favorably on the effect of combined non-specific protein and specific antisyphilitic medication in reducing presistently positive Wassermann reactions and Fischer[5] has especially advocated this type of treatment in paresis and tabes dorsalis, which he designates as "phlogistic therapy."

More recently, Ahlswede[6] has found that intragluteal injections of 10 c.c. of a sterile milk protein solution at intervals of about ten days were useful as supplements to specific spirocheticide therapy in the way of general non-specific protein stimulation of the body. Greenbaum and Wright[7] have likewise observed that intramuscular injections of 5 to 6 c.c. of a 4 per cent. suspension of milk proteins every two to three days for six injections were alone capable of influencing tertiary skin and mucous membrane lesions, bringing about their resolution and disappearance unaided by specific medication. Starcke[8] has recently described a method for the treatment of paresis consisting of the intramuscular injection of common boiled milk in the morning which he states produced a mild fever by evening when massage is given and neoarsphenamin administered.

Muller and Pfanner[9] have recorded a case of syphilis refractory to specific treatment alone that yielded promptly to combined injections of *luetin* and neoarsphenamin. Some years ago Dr. Schamberg and I treated a series of Wassermann-fast cases of syphilis with combined injections of *luetin* (subcutaneously) and arsphenamin (intravenously) and gathered the impression that the results were better than observed with injections of arsphenamin alone; unfortunately, our supply of *luetin* at the time did not permit a sufficiently extensive trial to warrant a more definite statement. Finally it may be stated that a rather large literature has accumulated upon the treatment of paresis in Europe by inoculations of living tertian malarial parasites or the spirochetes of relapsing fever, the paroxysms being found distinctly helpful in the treatment, along with specific medication, as discussed more fully in Chapter LXXVI.

Of course, the treatment of paresis with malarial inoculation may not be a form of non-specific medication in the usual meaning of the term. Ordinarily we regard injections of milk, peptone and other protein agents as aiding in the treatment of infectious diseases by producing fever, leukocytosis and increasing the enzymes and antibodies of the blood and fixed tissues. Malaria produces fever but scarcely any leukocytosis; but similar reactions produced by injections of milk, protein, etc., have not given as good clinical results; neither have inoculations with *Sp. obermeyerei* (relapsing fever), suggesting that the living malarial protozoa furnish something inimical to syphilis by a mechanism not understood at present and analogous to the effects of erysipelas and other streptococcus toxins (as contained in Coley's fluid) upon sarcomata.

Value of Non-specific Protein Therapy in Syphilis.—No one, however, would seriously consider depending upon non-specific agents alone for the treatment of syphilis. From experiments on rabbits I am convinced that injections of milk, milk proteins, peptone and *luetin* have no effects upon the spirochetes in the way of direct spirocheticidal activities; Greenbaum and Wright have also observed that intramuscular injections of milk proteins were practically without curative effects in acute early syphilis. *On the contrary, injections of non-specific protein agents may actually stimulate spirochetic activity,* as I have observed in some instances of scrotal and testicular syphilis of rabbits; for this reason I believe that *when these agents are employed in the treatment of human syphilis that there should be the simultaneous administration of spirocheticidal drugs,* just as in the case of the iodids, not only to secure the maximum therapeutic effects of the latter, but to prevent exacerbation and dissemination of syphilis as well. These agents may stimulate immunological processes in syphilis and especially proteolytic activity in organized syphilitic exudates, as shown by their autolysis in a manner similar to the action of the iodids. It appears that the effects are due in part to the vascular changes produced embracing congestion and increased capillary permeability. I am particularly interested in the possibilities of subcutaneous injections of *luetin* in this connection by reason of the possible allergic vascular changes that may be produced and thereby increasing the access of spirocheticide agents and enzymes to the infected tissues. Enough has already been done to show that carefully chosen and skilfully administered non-specific protein agents may be helpful in the treatment of some cases of chronic syphilis as adjuvants in treatment and especially in neurosyphilis; indeed, it is quite probable that the increased curative activity of arsphenamin and its congeners by subcutaneous or intramuscular injection, as compared with intravenous injection, may be due in part at least to the effects of local tissue necrosis releasing stimulating protein agents and the same may be true of intramuscular injections of mercury and bismuth. I have observed clinically that this form of adjuvant non-specific protein therapy was particularly useful in the treatment of chronic syphilis accompanied by debilitated states and especially tabes dorsalis; the nutrition has appeared to be improved with an increase of weight, erythrocytes and hemoglobin, to which further reference will be made in Chapter LXXVI, where the literature is reviewed with more detail.

Administration of Non-specific Protein Agents in the Treatment of Syphilis.—Up to the present time this form of therapy in syphilis has been largely confined to paresis and tabes dorsalis. Various agents have been employed as mentioned above. Fischer has employed plant albumins rich in nucleoproteins broken down into polypeptids ("phlogetan"); Ahlswede has employed milk proteins as likewise Greenbaum and Wright. The preparation should not produce too sudden or severe reaction and too many injections should be avoided in order to avoid a "proteinogenous tiring" or "proteinogenous cachexia" from over stimulation.

Fischer advises that an energetic course of specific medication should precede the non-specific treatment but Ahlswede apparently favors mild non-specific medication first to be followed by specific medication with iodin and bismuth preparations. In paresis he injects 1 c.c. of a 10 per cent. solution of milk proteins intramuscularly and states "if the increase of the leucocytes, the increase of the red cells and of the hemoglobin content, the increase of the blood platelets and of the sinking rapidity of the erythrocytes point to a strong reaction, the dose administered may be repeated after 3 to 4 days. Should no reaction occur the initial dose would have to be increased.

If the initial reaction is very strong it is advisable to choose a longer interval between the following injection, and also to reduce this second dose. As a rule five injections will prove sufficient (Fischer who uses "phlogetan" injects up to 10 c.c.). Treatment must be interrupted in all cases in which a sudden drop of the leucocytes and the platelets takes place. During the intervals, between the last (fourth and fifth) injections I advise beginning internal medication of iodine and bismuth injections, which should be carried on for a few weeks under permanent control of the blood picture. After 4 to 8 weeks' interval, according to the general constitution of the patient, a second "course" should begin with or preferably without specific simultaneous medication. These "courses" should follow one another in a regular sequence till the clinical improvement is obtained."

Intramuscular injections of milk (boiled for ten minutes and cooled) may be employed in doses of 1 to 10 c.c.; for sharper reactions vaccines of colon or typhoid bacilli may be injected intravenously in dose of approximately 100,000,000 organisms or sterile 2 per cent. solutions of peptone may be administered by slow intravenous injection in dose of 1 to 3 c.c. My own experience has been limited to injections of milk in dose of 2 to 5 c. c. for the purpose of producing mild stimulation in the treatment of undernourished tabetics.

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CHAPTER LXIX

HYGIENE AND GENERAL TREATMENT OF SYPHILIS

While specific medication in syphilis is naturally of first importance, general or auxiliary treatment and hygiene should not be neglected, and at the present time they do not receive the same attention as formerly when the mercurials and iodids were relied upon exclusively for specific treatment. It is necessary to inquire into the general health of patients; to observe their temperament and pay due attention to their general hygiene as the details of their mode of living, diet, occupation, the time devoted to exercise and sleep, etc. We should make our patients understand the necessity of a quiet and regular life, free of excesses of any kind. In certain nervous patients, especially women, these auxiliary medications are sometimes of great importance, the state of the functions and general health requiring supervision and treatment as well as the syphilitic infection.

General Hygiene.—Not infrequently the syphilitic requires advice on such subjects as sufficient and proper *exercise* to maintain physical fitness, plenty of *fresh air* and an adequate amount of *sleep*. The latter and a quiet normal life, free of undue physical and mental fatigue and excitement, are of

special importance in relation to invasion and infection of the central nervous system. As discussed in Chapter XXXVI mental strain and overwork undoubtedly favor infection of the central nervous system with *Sp. pallida*. It is well to impress the syphilitic patient that relapses and lesions may follow tissue injuries and shocks, undue exposure, or overstrain, physical or mental. The importance of avoiding these must be recognized, whether in private or business life.

Tabetics and others physically incapacitated derive great benefit from *massage* and *electricity* is frequently helpful and sometimes a valuable supplement to inunctions of mercury where nerve symptoms are prominent. The pains of tabes are sometimes relieved by either the static or high frequency currents, the former being more suitable when the blood pressure is low and the latter when it is raised.

Sexual excesses must be especially avoided, because of physical and mental depletion. During the infective stages sexual congress must, of course, be strictly forbidden.

Oral Hygiene.—The mouth requires special attention. Since the advent of the organic arsenicals in treatment, we are apt to over-look the importance of this subject, since stomatitis is not so likely to occur as in the days when mercury was employed more freely in treatment. But, as stated in Chapter LI, it is quite possible that some of the severe accidents of arsenical therapy are due to the lighting up focal infections, and the removal of tartar and stumps, the filling of cavities, etc., are of just as much importance in relation to the administration of arsphenamin and its substitutes as they are to mercury and bismuth, which are more likely to produce buccal accidents. It should be an invariable rule of practice to inspect the mouths of every syphilitic at frequent intervals and to insist upon proper dental supervision and care, with cleansing of the gums and teeth at least once or twice daily with good brushes and dentifrices. It is my practice to insist upon each patient consulting his dentist at the beginning of treatment and at intervals of six months thereafter during the years he is under observation and treatment. If open lesions are present in the mouth, I insist upon the patient informing his dentist of the syphilitic infection, as their character may not be recognized by him, to the possible danger of himself and others.

Mental Hygiene.—As so correctly stated by Fournier even in the days before the discovery of *Sp. pallida*, "an organ which is stimulated or overworked in a syphilitic is an organ which is menaced by syphilis, for stimulation tends to direct the disease to this organ. Cerebral syphilis is especially common after nervous and intellectual overwork, and still more so after excitement, late hours, and dissipation, the excitement of play, venereal excesses, etc."

As clearly shown by him clinically and confirmed in recent years by laboratory examinations, the nervous system is the most often affected by tertiary syphilis. I have already discussed in Chapter XXXVI the question of strains of *Sp. pallida* in reference to this subject and expressed the opinion that hereditary predisposition and neuropathic tendencies are more important than the question of strains of the organism. Individuals of these groups who have contracted syphilis should be cautioned at the outset against all possible causes of morbid stimulation of the nervous system, such as excesses of all kinds, and especially venereal excesses, intellectual work requiring continuous mental strain, fatigue, late hours, excessive emotion or business worry and even too violent bodily exercise. Indeed, what may be called *antinervous treatment* is especially indicated in individuals belonging to these two classes.

The majority of syphilitics are subject to mental depression and excessive worry regarding the disease and prognosis. Indeed, as Collins has so well stated, it may be impossible to cure the syphilitic "scars of the spirit," but these must be combated by suggestion and an optimistic outlook on the part of the physician. I firmly believe that we may honestly feel optimistic in the treatment of the majority of syphilitic patients and I do not hesitate to take sufficient time to express a full, candid and detailed description of the disease to meet individual cases and the general outlook. It is a mistake to make light of the patient's fears and symptoms, regardless of how trivial they may appear. To him or her they are of great importance and worthy of our sympathetic hearing and attention. On the other hand, the too cheerful and careless patient must be convinced of the seriousness of his condition. In other words no one method of handling this phase of the treatment is applicable to all patients; the physician must decide upon the proper attitude for each case, since the best method for one may be harmful for another.

Hydrotherapy.—This naturally brings up the subject of hydrotherapy in the treatment of syphilis of the nervous system as well as in relation to syphilis in general. Fournier was a warm advocate of this auxiliary treatment on account of its tonic and regulating action on the nervous system. He regarded its preventive action as equal to its curative effect in the treatment of affections of the nervous system and was in the habit of prescribing it to all syphilitic subjects who, by their hereditary predisposition or their personal tendencies, lead him to regard them as predisposed to the nervous manifestation of syphilis.

As stated in Chapter LIII, evidence at hand indicates that the ingestion of sulphur waters aids in protecting against the effects of mercurialism and, as mentioned in Chapter LI, it would appear that the administration of sodium thiosulphate and thiosinamin are useful in the treatment of arsphenamin dermatitis and allied reactions. Physicians of experience in certain watering places in Europe and America are frequently convinced that their patients are able to stand much larger doses of mercury by inunction than would otherwise be the case and especially elderly tabetics of poor nutrition; this is especially true at Aachen where the water contains small amounts of hydrogen sulphid, sodium sulphid, sodium iodid, sodium bromid as well as larger amounts of sodium chlorid, sodium carbonate and calcium carbonate. It is believed that the ingestion of this water results in the greater elimination of mercury with the promotion of diuresis and catharsis, resulting in an increased excretion of urea and sulphates in the urine and increased elimination of the skin with stimulation of the appetite, improvement of digestion, increased excretion of bile and general tonic effects.

Many syphilitics and especially the elderly who do not bear mercury well, fair much better at the Hot Springs of Arkansas in this country than in their homes; the good effects have been ascribed to a specific action of the water, both as baths and taken internally, to greater elimination, to stimulation, to the routine life, and to the effects of the salubrious climate. The alleged effects of the water have been ascribed to some peculiar chemical combination of the solids in solution, to electricity produced by chemical decomposition, to heat, and latterly to radio-activity. Loyd Thompson, practicing at the Hot Springs, does not believe that the waters have any specific influence upon *Sp. pallida* and has summarized his opinion[1] on their general effects as follows: "It is a fact, however, that patients bathing in these waters are able to take much more mercury without any untoward effects than others. It is furthermore highly probable that some of the bene-

fits derived are due to increased elimination of the products of catabolism. To these benefits derived from the increased ability to take mercury and the increased elimination must be added the benefits derived from the regular life the patient usually leads, the outdoor exercise, the freedom from business cares and worries, and finally, the fact that the average patient who comes to this or any other resort follows the physicians' directions more implicitly than when at home; in short, makes a business of getting well.

"While each case of syphilis is a law unto itself in regard to bathing as well as in other features, the following may be said to be fairly representative of the usual method of administering the baths to syphilitics in this resort:

"The patient is placed in a tub of hot water at a temperature of 35.5°C. to 38°C. (96°F. to 100°F.) where he remains from eight to fifteen minutes. When free diaphoresis is desired he is placed in a hot wet pack and covered with blankets for fifteen to thirty minutes, and when more profuse sweating is wanted the vapor cabinet is used for three to five minutes. Usually in the tub and in the pack the hot water is taken to drink to assist in promoting diaphoresis. Generally following the pack a shower and needle is given for two or three minutes beginning at a temperature of 38°C (100°F.) and being reduced to 32°C. or 27°C. (90°F. or 80°F.). Following this the patient sits or lies in the cooling room until cool and the skin is dry. Ordinarily the patient spends from one and a half to two and a half hours at the bath house."

Not all patients, however, can stand the expense of a sojourn from home and business, but it is sometimes possible to approximate this plan at their homes by using ordinary water with great benefit and especially in neuro-syphilitics of advanced years who are under weight, who need mercury and bismuth, but who do not tolerate sufficient doses of the drugs.

General Medication.—In addition to medicinal aid for the correction of constipation, the administration of stomachics and so-called "tonics" may be especially helpful when the appetite is poor and the patient under weight; Thompson prescribes the following:

℞ Tr. nuc. vom. ..... 45.0 c.c.
Tr. cardamon comp.
Glycerin ..... āā 90.0 c.c.
Aq. destil. ..... q.s. ad 240.0 c.c.

One to two teaspoonfuls in water are prescribed before each meal.

As a general rule the anemia of syphilis responds promptly to the administration of arsphenamin and its substitutes; iron is of no particular value. The organic arsenicals also frequently afford great relief to the emaciation and other untoward effects of mercurialization.

Diet.—In the fifteenth and sixteenth centuries, great attention was given the subject of diet in syphilis and other diseases. Depending upon the fads and fancies of the times patients were either starved or over-fed and the diets were made out with great care, so many foods being forbidden that one wonders how the victims survived. It is now well known that no foods are particularly favorable or unfavorable to syphilis, although, when mercury is being given, due attention must be paid to the diet to reduce to a minimum the chances of producing gastro-intestinal disturbances. Therefore, if the usual diet of the patient is good, it requires no change; if it is insufficient or defective, it requires suitable modification and if excessive, reduction to hygienic proportions. Excessively stout and florid individuals with high blood pressures generally overeat and exercise too little; these faults should receive attention. Thin, emaciated and anemic individ-

uals and especially elderly tabetics require overfeeding, rest and massage as auxiliaries to specific medication. In syphilitic nephritis and in gastric and liver syphilis, special attention must be given the diet as likewise syphilitic infants and children, the former sometimes presenting unusual difficulties when fed artificially.

When arsphenamin or one of its substitutes is being administered by intravenous injection, it is well for the stomach to be empty; the preceding meal should be light and taken at least two hours previously, to minimize the chances of producing nausea and vomiting. When intramuscular injection of sulpharsphenamin, bismuth or mercury are being given, these precautions are not important.

When patients show a tendency for developing diarrhea and colic after the administration of the arsenicals, mercurials or bismuth compounds, the diet should receive special attention; under such circumstances iced drinks, uncooked vegetables and fruits and especially salads may require reduction or omission, whereas milk and foods prepared of milk may be given more freely. Due care must be taken to exclude such foods for which the patient shows allergic hypersensitiveness or idiosyncrasy; women especially are apt to rebel against eggs.

Beverages.—Fournier has stated that alcohol is the born enemy of the syphilitic and constitutes a serious factor, both in a general way and in relation to certain special lesions, such as the dermatoses and manifestations of the nervous system. Most syphilologists warn against even small amounts of alcohol as being detrimental and especially moderate drinking all the time rather than a spree once or twice a year.

Constipation should always be corrected and the subject is worthy of special attention in women patients; practically all of the specific remedies are eliminated in part through the bile and intestinal canal and due care is required to prevent reabsorption. It is commonly stated that alcoholics are more susceptible to the untoward effects of arsphenamin and its substitutes, probably as a result of cardiovascular changes and especially of the vessels of the kidneys. Possibly, too, the metabolism of the alcoholic individual favors the production of toxic products of the drugs in the organs and tissues to a greater extent than in non-alcoholic persons.

As in everything else, however, common sense is required in giving advice on the use of alcoholic beverages. As a general rule they should be forbidden, but the elderly and undernourished may require small amounts of wine or other light alcoholic beverages once or twice a day at meal times. I have not observed any harm from alcoholic beverages in strict moderation under such circumstances.

Coffee and tea in moderate amounts are not harmful provided gastro-intestinal disturbances are absent. An excess of either, however, and especially in cases showing nervous manifestations, should be forbidden. As a general rule the free and liberal use of water, milk, butter-milk, etc., should be encouraged.

Tobacco.—Smoking, and especially excessive smoking of strong tobaccos, is harmful for many syphilitics, as shown by the high incidence of tongue and buccal lesions among men as compared with women. When lesions are present in the mouth, tongue or throat, I believe that smoking and chewing should be absolutely forbidden or greatly curtailed. Given a syphilitic glossitis plus smoking and the tissues are well prepared for cancer. In the absence of manifest lesions, moderate smoking may be allowed, but I believe it is always the duty of the physician to carefully explain to the patient that smoking greatly increases the chances of producing lesions of the lip, tongue

and throat that may eventually prove of serious import. This is due to the fact that tissue irritation and injury always predisposes to the localization and multiplication of *Sp. pallida.*

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CHAPTER LXX

CHEMICAL PROPHYLAXIS OF SYPHILIS

With a disease as infectious as syphilis, it is not surprising that many methods have been advocated for reducing its incidence. These may be divided into those that may be taken by the individual, and those that may be taken by the community. A great deal of discussion has been raised on the moral aspects of methods for the prophylaxis of syphilis on the basis that to make exposure safe is to increase immorality, but I shall not enter upon this subject since very complete and splendid discussions have been published by Fournier[1] and more recently by Vedder[2] and Stokes.[3] Neither shall I consume space in a discussion of community or public measures for the suppression of syphilis and other venereal diseases by the suppression or regulation of prostitution, etc.; nor upon the use of circumcision or mechanical devices for individual prophylaxis, but devote myself to a consideration of the subject of chemical or medicinal prophylaxis against genital and extragenital syphilitic infection to be applied within the first few hours after contamination and possible infection. The subject of abortive treatment, that is, treatment in the incubation period between the time of infection (exposure) and the development of the chancre, is considered in the succeeding chapter.

Individual Chemical Prophylaxis of Syphilis and Other Venereal Diseases. Individual chemical prophylaxis refers to the use of various substances by the individual before or after sexual contact or extragenital contamination for the prevention of infection by the destruction of spirochetes deposited on the skin or mucous membranes or in abrasions of these before infection has occurred or within a few hours thereafter. Probably one of the earliest attempts in this field was made by Fallopius, published in his book, "De Morbo Gallico," printed in 1564, and recently reviewed by Lane.[4] Fallopius advised washing the parts immediately after exposure and placing over the glans penis a piece of cloth impregnated with gentian, santal wood, aloes and other substances and claims that he tested this in one thousand, one hundred cases with no infection! As stated by Lane, some of his successors in syphilology have been inclined to question his veracity and that it is safe to assume at least that he probably did not have one of our modern, efficient follow-up systems at his disposal!

According to LePileur,[5] Agato at about the same time used fumigations of cinnabar and Desault is said to have advocated the use of mercurial ointment in 1733, reasoning that if mercury cures syphilis it ought to prevent it as well. During the following 160 years, these methods do not appear to have commanded much attention; in 1899, however, Behrmann,[6] Cohn,[7] Richter,[8] Loeb,[9] Guiard[10] and others greatly renewed interest in the subject by the reports of uniform success among men following applications of mercurial ointment, bichlorid of mercury, etc., to the penis immediately after coitus with syphilitic women.

Prophylaxis in Experimental Syphilis.—But after the discovery in 1902 by Metchnikoff and Roux,[11] that syphilis could be transmitted to chimpanzees, interest in the subject of prophylaxis was greatly renewed by their experiments employing mercurial ointment and later a salve of 10 parts of calomel and 20 parts of lanolin. In 1906 they reported[12] that, having tried twelve experiments on monkeys with uniformly successful results, they performed an experiment on a volunteer medical student (M. Maissonneuve) with complete success. Naturally these experiments attracted wide attention and in most quarters there was a tendency to accept them as a solution of the problem of the prophylaxis against syphilis. Levy-Bing,[13] however, claimed that the evidence was not conclusive and recorded a failure in a case of Gaucher's and additional occasional failures were soon recorded, the most severe critic of calomel ointment being Neisser,[14] who conducted a very large series of experiments upon apes with the result that inunctions and washes did not regularly prevent infection. Neisser showed, however, that simple washing with water for fifteen minutes prevented the infection of two apes and success was also observed with pure phenol, 1:2000 to 1:3000 bichlorid, 5-per cent. silver nitrate and 10- to 30-per cent. suspensions of calomel in soap, vaseline and saline solution; failures followed the use of iodoform, mercurial ointment, bichlorid 1:1000 and 10-per cent. suspensions of calomel. Metchnikoff,[15] however, promptly pointed out that Neisser's failures were with 10-per cent. calomel preparations and not with the 33-per cent. ointment which he recommended; in a second series of experiments with seven monkeys, he showed that the calomel must be at least of 25-per cent. strength and recommended 33-per cent., stating that "experiments on the preventive action of mercurial ointment on monkeys are sufficiently numerous and conclusive to form a basis of a prophylaxis against syphilis." Vorberg[16] believed that Neisser's failures may have been due to too deep scarifications, which may readily prevent access of the calomel to the spirochetes. However, Neisser was not opposed to the method; indeed, he was a warm advocate of it, but maintained, after another very extensive series of experiments with apes in collaboration with Seibert,[17] that bichlorid of mercury was superior to calomel ointment because he found calomel less spirocheticidal *in vitro*, and that its activity was much reduced because of its incorporation in a fatty base. Neisser recommended a mixture of starch, tragacanth, gelatin and mercuric chlorid, because he believed it a more powerful spirocheticide, more likely to adhere to damp and abraded surfaces and more successful in preventing infection. This naturally led to considerable investigation on the comparative bactericidal activity of various germicides in solution and in salves, with the general result that the former were found more effective, but the results did not apply to calomel which is practically insoluble in both. As previously stated, Metchnikoff prepared his ointment by mixing 10 parts of calomel with 20 parts of ordinary lanolin (not anhydrous); in the U. S. Army the following mixture has been employed:

Calomel	30 parts
Benzoinated lard	65 parts.
White wax	5 parts

Recently Nichols and Walker,[18] working with inoculated rabbits, found that this ointment proved efficacious up to eight hours after inoculation with syphilis and that no marked difference appeared between the action of calomel in a base of lanolin and vaseline and in a base of benzoinated lard and wax.

Animal experiments have, therefore, conclusively proved the prophylactic value of calomel ointment and despite Neisser's vigorous protests (he appeared to be somewhat biased in his opinions), it has been employed much more widely than solutions of bichlorid or other substances, notably quinin ointment advocated by Schereschewsky[19] and others, dusting powders of sodium and potassium tartrobismuthate by Levaditi,[20] etc. As stated by Vedder, bichlorid solutions are open to serious objection on the basis of high toxicity and furthermore, they dry so quickly that their spirocheticidal action is probably of briefer duration than is the case with calomel ointment. It is true that Schumacher[21] and others have recently sought again to discredit Metchnikoff's claims, but the evidence from animal experiments is overwhelmingly convincing. In Chapter XXVIII I have shown the high spirocheticidal activity of mercury *in vitro* and the experiments of Neisser and Seibert, consisting of mixing emulsions of syphilitic spleen, testicles and bone-marrow of apes with various solutions of bichlorid and calomel, followed by inoculating the material into apes to determine the degree of spirocheticidal activity *in vitro*, have likewise shown the powerful effects of these substances when brought in contact with virulent spirochetes. In their experiments water and salt solution alone were without effect, but 1:5,000 to 1:10,000 solutions of bichlorid and 10-per cent. suspensions of calomel in saline and oil were generally effective when allowed to remain in contact with the spirochetes for at least fifteen minutes before inoculation. My experiments employing rabbits have yielded similar results and, since the publication of the article by Nichols and Walker, I have been able to confirm their work on the prophylaxis of syphilis in rabbits with calomel ointment in every essential particular. It is true that failures have occurred, but I believe these were due to too deep

scarifications, excessive oozing and bleeding and such fortuitous circumstances preventing the contact of calomel with the organisms; personally, I am convinced of the extremely high prophylactic activity of 33-per cent. calomel ointment in experimental infections when applied within four hours after inoculation. Furthermore, a very large literature has accumulated on the subject as applied to human beings exposed to syphilis, which is likewise convincing despite isolated failures.

Prophylaxis in Human Syphilis.—It is not profitable, however, to attempt analyzing all of this literature here, because many of the reports will not stand an impartial scientific scrutiny of the results, although their authors were personally convinced of the correctness of their results and conclusions. Metchnikoff,[22] Wolbarst[23] and others, however, have reported individual cases of protection almost as convincing as experiments with the lower animals, although apparent failures have likewise been reported by Butte,[24] Gaucher,[25] Carle,[26] Sutton[27] and others. Probably the statistics furnished by Army and Navy surgeons are the most valuable of all, because it is possible to study the subject in these services under much better conditions than in civilian practice. The reports of Acevedo[28] in the Chilean navy, Feistimantel[29] in the Hungarian army, Tandler[30] and Seibert[31] in the German army and navy, Michels,[32] Wickes,[33] Davidson,[34] Ledbetter,[35] Ashford,[36] Cottle,[37] Riggs,[38] Exner,[39] Reynolds and Wolfe[40] and others in the United States army and navy, have shown in a convincing manner that syphilis undoubtedly can be prevented by the use of various prophylactics, and particularly by the use of 33-per cent. calomel ointment.

It has been noted that a delay of more than four hours in applying prophylaxis results in a considerable jump in the incidence of infections although the incidence after a delay of even 10 hours was doubtless much less than among cases receiving no prophylactic treatment at all. Probably the best statistics have been furnished by Colonel Wolfe, from Fort McKinley, P. I., for the period from January, 1913 to August, 1915; during this time treatments were given 19,465 times with but 2 cases of primary syphilis, whereas 99 cases developed among men who did not take the calomel prophylactic.

Limitations and Value of Chemical Prophylaxis in Human Syphilis.—I believe we may conclude from the experimental and practical work with venereal prophylaxis that syphilis, gonorrhea and chancroid undoubtedly can be prevented in the majority of instances by the use of appropriate prophylactic measures. Calomel is highly bactericidal for the gonococcus and *B. ducrey* (chancroid) as well as for *Sp. pallida;* the application of 33-per cent. ointment may, therefore, protect against both syphilis and chancroids, while a suitable silver salt like argyrol or protargol, is injected into the urethra for the destruction of gonococci. In practice it cannot be expected that their use will be invariably successful, but it appears that when properly applied within one to four hours after exposure that they will prevent the great majority of syphilitic infections. Experience in the army and navy has shown that good results can only be expected from the use of prophylactic measures when the medicinals are provided and when medical and other authorities unite to instruct the men in their value and the method of use.

One may very properly inquire if calomel ointment or other medicinal prophylactics destroy all spirochetes at the site of application and thereby actually and truly prevent syphilitic infection, in the true sense of the term, or merely reduce them in numbers or virulence to such an extent that a primary lesion is not produced or escapes detection while the lymphatic system is infected with the subsequent development of lesions. I am confident that prophylaxis can be made complete in the strictest sense, in

rabbits at least, because frequent tests with excised lymph-glands have yielded negative results when emulsions were injected into the testes of fresh animals; furthermore, most clinical observers, especially in the army and navy, have had a sufficient number of men under observation along with repeated Wassermann tests with absolutely no clinical or serological evidences of infection, to warrant the statement that these measures may be relied upon *when properly applied* as truly prophylactic for syphilis in the strictest application of the term.

One of the most important phases is thorough cleansing of the parts and the application of the prophylactic within an hour after exposure; the lapse of more than four hours reduces the chances of success quite materially. This is a point worthy of considerable emphasis, as not infrequently men delay in applying the prophylactic too long or repeatedly expose themselves over a period of six to twelve hours or more before applying the measures after the last exposure which reduces the chances of affording protection after the first exposure.

The method has not been extensively applied to women because of the anatomical difficulties, but there is no reason why it should not be tried when syphilitic infection is suspected; failure, however, occurs much more frequently than in men.

The method has likewise failed in deep inoculations with syphilitic virus as the pricking of the fingers with needles, etc., by surgeons. Under such circumstances very thorough cleansing and application of calomel ointment may be tried, but when there is great danger of infection and much worry, I believe it is well to follow up these with at least three injections of neoarsphenamin, which I have discussed in the succeeding chapter with more detail.

Furthermore, *the prophylactic is only of value to the parts treated.* I have known two men, apparently escaping genital infections as the result of calomel prophylaxis, who contracted syphilis of the lips from kissing their sexual partners who had mucous patches in their mouths. It is important to remember in this connection, that the prophylactic should be applied to the lips as well, after contact with a syphilitic with open lesions about the lips or in the mouth.

Sexual contact with a Wassermann positive woman with chronic, latent syphilis but without open lesions, is not likely to be followed by infection, unless frequently repeated, although small abrasions may be present in the vaginal and cervical mucous membranes easily escaping detection. For this reason prophylaxis should always be resorted to if possible, regardless of the question of probable absence of open lesions.

Men with chronic syphilis with no open penile lesions are not nearly so infectious as when a chancre is present, although the semen may be infectious. It is not to be forgotten, however, that women may escape infection after sexual contact with men having penile chancres, as recently reported by Troisfontaines,[41] but I always suspect in such cases that the woman may be already syphilitic with the possible protection against superinfection given by the presence of living spirochetes in her tissues.

Method of Applying Genital Prophylaxis in Men.—(1) As shown by Worms[42] and others, the application of prophylactic before intercourse is not as efficacious as post-coital application. However, preliminary application of the ointment is advisable, if for no other reason than that lubrication reduces the chances of producing abrasions.

(2) As soon as possible after coitus the parts should be thoroughly washed with soap and water. As shown by Reasoner,[43] a solution of soap of even

a considerable degree of dilution, and a lather as used in shaving, may be sufficient for destroying *Sp. pallida* and it is probable that chancroidal infection may be also avoided by the same means.

(3) The parts may now be rinsed with water followed by thorough cleansing with a 1:1000 solution of bichlorid of mercury in water, although this step is not absolutely necessary and should not be advised to ignorant and careless individuals on account of the dangers from poisoning if bichlorid is swallowed; there is no danger, however, from the local application of such a solution, but stronger solutions are unnecessary and more likely to produce irritation.

(4) The parts are dried and about 1 dram of 33-per cent. calomel ointment well rubbed in, with particular attention to the glans, corona and prepuce, but neglecting no part of the penis, anterior portion of the scrotum and the pubis as well (aids in preventing pediculosis). At least ten minutes should be consumed in making the application and the parts should now be covered in order to protect the clothes, leaving the mercury to act, for at least twelve hours on any organisms that may remain.

As stated above, the base employed for preparing the calomel ointment does not appear to be as important as originally believed, and it is well to use Metchnikoff's formula:

Calomel	10 gm.
Lanolin	20 gm.

Anhydrous lanolin should not be used and it is important to have a *thorough mixture of the calomel and base.*

Other substances may be used but calomel is probably best. For example, Lenz[44] has recently advised a 0.3-per cent. (1:300) of bichlorid ointment. In experimental syphilis of rabbits I have had uniform success with applications of a tooth paste described in Chapter XXXI for the treatment of spirochetic and bacterial gingivitis, containing 1 per cent. arsphenamin and 0.5 per cent. mercurophen or bichlorid of mercury. An extensive series of experiments with syphilitic rabbits has convinced me that the application of this mixture is as efficacious as 33-per cent. calomel ointment, although it has the disadvantage of being yellowish in color and thereby more likely to stain the underclothing.

(5) For the prevention of gonorrhea, an intra-urethral injection of 10-per cent. argyrol may be given; recently, however, I have advised 10 per cent. neosilvol because it is colorless and does not have the objectionable brown discoloration of the former. In some prophylactic packets it is advised to inject a portion of the same ointment as is used for application to the external parts.

(6) At least two weeks must elapse with absolutely no signs of a discharge or sore before the individual may regard himself as having escaped gonococcus and chancroidal infections and at least six additional weeks must elapse without a sore before he may feel assured of having escaped syphilis, although the usual period of incubation is two to four weeks.

Method of Applying Genital Prophylaxis in Women.—As previously stated, prophylaxis among women is much more difficult and uncertain than among men and especially from the standpoint of vaginal and cervical infections; furthermore, a woman cannot ordinarily give herself an intraurethral injection for protection against gonorrhea.

Before coitus calomel ointment should be applied with special attention to the fourchet and a small amount introduced into the vagina. As soon as possible after coitus the parts should be thoroughly washed with soap and

water and a cleansing vaginal douche taken preferably of a quart of warm water to which has been added a teaspoonful of lysol. This should be followed by a douche of one quart of 1:2000 bichlorid solution, the individual being on her back, the nozzle well introduced and the solution held in contact with the cervix and vaginal walls for at least 5 minutes. After this the parts should be dried and calomel ointment thoroughly applied for ten minutes to the external parts, including the anus and pubis and particularly the labia minora and fourchet. A napkin should be worn for at least twelve hours before the parts are cleansed.

Since these measures may fail to prevent cervical infections, although very efficacious against infection of the external parts, it is well when connection has occurred with a man with a penile syphilitic lesion, to resort to abortive treatment in addition, which consists of several intravenous injections of neoarsphenamin, as described in the succeeding chapter.

Prophylaxis of Extragenital Syphilis.—Surgeons, obstetricians and midwives are especially exposed to the dangers of extragenital infections. I have seen several cases among surgeons after tonsillectomies upon syphilitics with open lesions in the mouth and fauces. I have never seen or heard of a case among pathologists due to necropsy inoculations; not among serologists as a result of drawing serum or blood from syphilitics into the mouth.

Needle-pricks and cuts should be encouraged to bleed, followed by immersion in hot 1:1000 bichlorid for at least ten minutes; after drying, 33-per cent. calomel ointment should be rubbed in for at least ten minutes and repeated every six hours for three applications, the excess ointment being left in contact for at least twenty-four hours. I believe these measures are sufficient, but if the chances of infection are great, it will be well to follow up with an intravenous injection of 0.6 gm. of neoarsphenamin every other day for two or three doses.

After exposure by kissing a syphilitic with a chancre on the lip or with mucous patches in the mouth, the lips, cheeks or other contaminated places should be washed as soon as possible with warm water and soap, dried and anointed for ten minutes with 33-per cent. calomel ointment, the excess being left on for at least twelve hours. The mouth may be rinsed out with 1:2000 solution of bichlorid several times, each mouthful being held for at least two minutes with due care against swallowing, the final washing being with water or dilute alcohol to remove the metallic taste.

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CHAPTER LXXI

ABORTIVE TREATMENT OF SYPHILIS

As discussed in the preceeding chapter, it is possible to destroy *Sp. pallida* by various chemical substances including plenty of soap, if the applications are made within one to four hours after the parasites have gained access to the superficial tissues in either genital or extragenital contamination or infection; this is properly designated as *chemical or medicinal prophylaxis*. Not infrequently, however, prophylactic measures cannot be taken in time, are very imperfect or the individual does not know for one or more days that exposure to a syphilitic individual has taken place, all of these being especially true of women. If more than ten or twelve hours have elapsed and certainly if forty-eight hours have elapsed since exposure to open syphilitic lesions, it is highly probable that the application of 33 per cent. calomel ointment will prove ineffective for prophylaxis, since the spirochetes have in all probability multiplied to some extent, invaded the lymph spaces and even the neighboring lymphatics. At least, Brown and Pearce[1] have found that rabbits inoculated intratesticularly and scrotally may show the presence of spirochetes in the neighboring lymphatic glands forty-eight hours later and the same may occur in human beings. But there are no signs of infection beyond a possible abrasion and the primary sore or chancre does not appear for about ten days to two months, although the usual period of incubation is two to four weeks.

It is possible, however, to bring about a destruction of the spirochetes during this time and thereby prevent the development of the chancre and other lesions of syphilis; this may be properly designated as the *abortive treatment of syphilis* and constitutes the subject for consideration in this chapter.

The term is not infrequently employed, however, and especially by German writers, to designate the intensive treatment of syphilis during the primary or chancre stage, to abort the disease before secondary and later lesions appear; but in my opinion this is not the proper use of the phrase.

Abortive Treatment of Experimental Syphilis.—Curiously, but very little experimental work has been done on this important subject, although a number of clinical reports have appeared since 1919, when Taege[2] advocated the administration of three doses of sodium arsphenamin within a period of twenty-four hours for the abortive treatment of syphilis. Darier[3] reports that Dr. Magian of Paris experimented upon himself by inoculating his forearm from a primary lesion swarming with *Sp. pallida*, followed one hour later by the intravenous injection of 0.6 gram of arsphenamin. On subsequent observations over a period of 11 months no lesion appeared at the site of inoculation and the complement-fixation test remained persistently negative. Levaditi and Navarro-Martin[4] record a second human experiment in a young man, who 16 hours after inoculation, took by ingestion two grams of the sodium salt of oxy-aminophenyl arsenic acid; during a 47-day period of observation no lesions of syphilis developed.

The only reference to animal experiments which I have been able to find is that of Mibelli,[5] who states that Vecchia found that rabbits inoculated with syphilis do not develop the disease if neoarsphenamin in dose of 0.01 gram per kilo is given as late as 4 days after inoculation.

While it is impossible to duplicate exactly in rabbits the conditions pertaining to human infections, yet I believe they are of great importance in the study of this problem. With human volunteers there is always the possibility of latent unrecognized syphilis which may confer protection against superinfection with the result that failure to develop a lesion at the site of inoculation is ascribed to the curative activity of the drug administered; with rabbits this is easily avoided and numerous untreated controls may be included. Furthermore, with human beings treated after sexual exposure to syphilitics, the question of infection is assumed and cannot be proven; with rabbits the question of infection can always be definite and decided. With human beings the question of infection can only be decided by prolonged observation for the development of lesions and positive Wassermann reactions and it may be possible that primary lesions are not produced although infection of the lymphatics may occur; in rabbits the question can be decided by removing the regional lymph-glands and inoculating them into fresh animals. For these reasons, therefore, rabbit tests would appear to be of value in the study of this very important and practical subject.

During the past year, Greenbaum and Harkins[5a] made such a study in my laboratory. Rabbits were inoculated intratesticularly, as described in Chapter XXI, with a virulent strain of *Sp. pallida*, producing almost 100 per cent. positive infections in untreated controls. Ordinarily 0.010 gram of arsphenamin (disodium salt) produces the complete cure of such animals including sterilization of the lymph-glands, when the injection is given after a testicular lesion has developed (usually 3 to 6 weeks after inoculation); but this dose, which corresponds to 0.6 gram arsphenamin per 60 kilograms or 132 pounds, did not prevent the development of syphilis in rabbits when given three and twenty-four hours after inoculation. Even a dose three times larger, namely, 0.03 gram per kilo corresponding to 1.8 gram per 60 kilograms or 132 pounds, did not prevent syphilis when given three hours and twenty-four hours after inoculation. However, three daily doses of 0.010 gram per kilo were effective in that lesions did not develop and the lymph-glands were sterile.

Similar results were observed with neoarsphenamin. Ordinarily a dose of 0.015 gram of the best products given to rabbits with acute testicular lesions, results in their cure with sterilization of the lymph-glands. However, this dose, which corresponds to 0.9 gram per 60 kilograms or 132 pounds, given to rabbits three and twenty-four hours after inoculation did not prevent the development of lesions; even a dose three times larger, namely, 0.045 gram per kilo corresponding to 2.7 gram per 60 kilograms or 132 pounds, given three hours and twenty-four hours after inoculation failed to prevent the development of testicular lesions although three daily doses of 0.015 gram per kilo each were completely effective.

Sulpharsphenamin of the best grades is generally curative for rabbit testicular syphilis in dose of about 0.020 gram per kilo. Rabbits treated with a single dose of 0.015 gram per kilo, corresponding to 0.6 gram per 60 kilograms or 132 pounds, one and twenty-four hours after inoculation, developed testicular syphilis. Even doses three times larger, namely, 0.045 gram per kilo, corresponding to 2.7 gram per 60 kilograms or 132 pounds, failed to

prevent the development of testicular lesions when given one and twenty-four hours after inoculation. However, three daily doses of 0.015 gram each were effective.

These results were, therefore, very interesting and instructive. One would naturally suppose that smaller amounts of the organic arsenicals would suffice for the destruction of *Sp. pallida* given three to twenty-four hours after their injection into the tissues, than for the cure of well developed lesions containing thousands more of the parasites. But apparently such is not the case. If the inoculations had been given by abrasions of the scrotal skin, one may surmise that drugs introduced into the blood are eliminated too rapidly for them to reach parasites so superficially placed, but in Greenbaum and Harkin's tests the parasites were placed in the substance of the testicle. Naturally this raises the question of why arsphenamin, neoarsphenamin and sulpharsphenamin were found so much more effective in the cure of three to six weeks' developed testicular lesions than for three to twenty-four hour inoculations with little or no tissue changes. One may speculate at considerable length, since it brings up the question of the part played by the tissue reaction in defense and recovery from spirochetic infection. This subject has been already discussed in Chapter XXXVII, in which it is stated that the local tissue reactions, like the chancre, play a very important part in building up resistance to the progress of syphilitic infection and that anything preventing this reaction which fails at the same time to destroy all spirochetes, only hastens the multiplication and migration of the surviving organisms. The experiments have likewise shown the futility of single doses; at least three full-sized doses were required. I will, however, delay a further discussion of the subject in relation to the prophylaxis of human syphilis until the available literature has been reviewed; I hope it is apparent, however, that the subject is not as simple as one would at first suppose and furthermore, that it is of tremendous practical importance to individuals relying upon abortive treatment for escaping serious syphilitic infection.

Abortive Treatment of Human Syphilis.—As previously stated, Taege appears to have been first in advocating the abortive treatment of syphilis of human beings and in replying to Riecke,[6] who objected to the use of arsphenamin until the diagnosis of syphilis could be established, which he asserted was early enough done in the chancre stage of the disease to institute abortive treatment, states that the diagnosis of syphilis is not always possible early enough to adopt abortive treatment. He maintained that this was especially true in women, for whom the abortive treatment was peculiarly adapted.

Fournier and Guenot[7] report on the abortive treatment of syphilis by arsphenamin used during the incubation period. Forty women had been exposed to infection by sexual contact with syphilitics having genital lesions. The men were all examined, and the presence of *Sp. pallida* demonstrated by the dark field. The Wassermann reaction was positive in all except five, who had had the chancre less than ten days. The period since exposure for the women was from a few days to three weeks. The women were free of all suspicious lesions, the history did not lead to the suspicion of a previous infection, and the Wassermann reaction was negative in all. Not one had exercised any preventive measures. Five additional women, similarly exposed but who refused all treatment, were later attacked by syphilis.

The forty women were treated by injections of arsphenamin (a total of from 1 to 1.20 gm.), of neoarsphenamin (a total of from 1.2 to 2 gm.), or of an arsphenamin substitute. Not one of them ever showed any syphilitic lesions, and the Wassermann reactions of all have remained negative. Twenty of the women have been under observation over three years. One of the women, who after this treatment continued to receive her sexual partner, who had mucous patches for which he did not take treatment, contracted a chancre of the face.

Lacapere and Laurent[8] have reported the following four interesting observations on the abortive treatment of syphilis:

Of three officers who had intercourse with one woman who presented a pigmentary syphilis and numerous mucous patches of the mouth and vulva, two received a dose of neoarsphenamin (amount not mentioned) intravenously and remained unscathed. The third preferred to take his chances with infection and after the usual incubation period, presented the primary sore of syphilis.

The wife of an officer, who had connection with her despite his having a chancre and a positive Wassermann reaction, was given three injections of neoarsphenamin, in doses of 0.15, 0.3, and 0.45 gm., at intervals of six days. She never presented any lesions, and her Wassermann reaction remained persistently negative.

A young man with erosive syphilitic lesions of the glans and prepuce had repeated contacts each night for fifteen days with a young woman who presented no lesions, no history

of antecedent syphilis, and a negative Wassermann reaction. She was given eight injections (dosage not mentioned) of neoarsphenamin and she has remained free of lesions, and Wassermann negative.

A nursing heredosyphilitic infant had exposed two wet-nurses to infection. One of the two later nursed another infant. About eleven days after the last nursing of the first infant she presented a lesion of the nipple, from which spirochetes were demonstrated by the dark field. The child she was then nursing was certainly not a heredosyphilitic, and the possibility of infection was certain. At intervals of five days, neoarsphenamin, in doses of 10, 15, 20, 40 and 50 mg., was given intravenously. Eighty-five days after the last feeding from the affected breast, no lesions had appeared in the child.

Michel and Goodman state that they have used arsphenamin in the prophylaxis of syphilis while in the tropics, where a large proportion of the promiscuous women were affected with syphilis. At that time the use of the drug was limited to men who had been exposed over twelve hours and frequently as long as three days previously. These men were given three doses of arsphenamin, a total of 0.9 gm., within forty-eight hours. Other patients were treated with arsphenamin who had lesions typical of the so called soft sore or chancroid, in whom it was not possible to demonstrate spirochetes by dark field examination. This group was treated with the idea of preventing the appearance of manifestations of a syphilitic infection which was still in its first incubation period. Most of these men were under observation only for a period long enough to determine that there no chancres formed at the close of the ordinary period of incubation time of syphilis, or, in a few instances, as long as six months later.

More recently thirty patients have undergone the prophylactic treatment with arsphenamin. The patients that have been selected for this mode of abortive treatment have been, in the main, those in whom it was definitely possible to make a diagnosis of active syphilis in the opposing partner. This is a modification of the "diagnosis by confrontation" of the older writers in syphilis. For example, when the diagnosis of active syphilis was made those likely to have been exposed to infection, usually the wife, and less frequently the husband, were examined. In the instances reported, they have been found free of lesions, but the possibility that they were in the primary incubation period of syphilis was so strong that, rather than wait for the appearance of symptoms clinically or serologically, arsphenamin injections were instituted.

The prophylactic doses have averaged 0.3 gm. of arsphenamin, and the number and interval of the injections have varied with the time since first exposure. In no case has this been less than three doses. They have not been consistent as to the interval of injections, varying from an injection every other day to once in five days, depending on the general physical condition of the patient. The women have been awarded the longer interval in the great majority of the cases. The patients treated in this manner have been under observation long enough to state that the primary incubation time passed without the appearance of primary lesions. No patient treated in this manner has developed a positive Wassermann reaction, although it was not possible to repeat the tests in every patient.

At the fifth Congress of the Northern Dermatological Society held in Stockholm in 1922, Heden[10] reported on 36 individuals who had had intercourse with syphilitics presenting open lesions and who had taken abortive treatment. Twenty of these cases had received neoarsphenamin and none developed lesions of syphilis over periods of observation ranging from 1½ months to 8½ years; all had been exposed 2 to 13 days (one case 3 weeks) before the injections were given. All escaped syphilis while of 36 additional control cases to whom abortive treatment was not given, 30 developed syphilis.

Hecht[11] reports on five cases, wives of men recently infected with syphilis, who were given abortive neoarsphenamin treatment (in dosage of 1.35 gm. in 10 days), and none of whom apparently developed syphilis. May[12] reports that prompt abortive treatment prevented the systematization of syphilis in two physicians, one whose eye was infected with syphilitic saliva and the other who cut his finger in puncturing a chancre. Favorable reports have also been made by Nicolan,[13] Golay,[14] Schmitzer,[15] Mibelli,[16] Gouin[17] and Rostenberg.[18] Simon[19] states that abortive treatment should only be given within 72 hours of exposure and not after this time for fear of only preventing the development of a chancre and giving a false sense of security. He advises at least three injections of neoarsphenamin in dose of 0.3 gm. every three days with a Wassermann test two weeks after the last dose and then monthly for at least four months. Schonfield[20] has suggested it as possibly worth while for prostitutes to receive a monthly injection of arsphenamin or neoarsphenamin for abortive treatment. Sutton[21] believes that when there is exposure to syphilis, even when the evidence of infection is presumptive, as in rape or when prophylaxis is neglected or delayed, abortive treatment consisting of injections of from 0.2 to 0.4 gm. of neoarsphenamin are worth while. He states that this has been apparently successful in two cases, one when a needle contaminated with the blood of a syphilitic was accidentally jabbed into a surgeon's forearm, and once when an assistant's eye and cheek were splashed

with blood; also in several additional cases when the hands were exposed in the handling of chancres and during operations on syphilitic patients. Martinotti,[22] however, is opposed to abortive treatment immediately after exposure, believing that it is better to wait for the development of the chancre and then to resort to intensive treatment.

Levaditi, Fournier and other French authors have reported the successful abortive treatment of syphilis in rabbits and man by the administration of stovarsol by mouth. This drug is given in dose of 4 tablets (0.25 gm. each) every morning before breakfast in a little water for 5 to 7 days; after intervals of 5 to 7 days the treatment is repeated so that the patients receive about 12 to 16 grams during the first month. In two men the development of syphilis is said to have been prevented, in spite of massive inoculation with spirochetes, by this drug. The number of persons who have been protected from infection by the drug in spite of sexual contact with a partner with florid syphilis is said to be considerable, but as stated by Bloch[23] and others the remedy is not a harmless one, is no more free from unpleasant or even injurious by-effects than its predecessors and the results are not yet sufficiently conclusive for using the drug in general practice.

Limitations and Practical Value of Abortive Treatment of Human Syphilis.—But it would appear that abortive treatment of human syphilis has been proven possible and to possess practical value under the following conditions:

(1) For individuals of either sex exposed sexually or extragenitally to active, open cases of syphilis when the prophylaxis described in the preceding chapter has not been applied within four hours of exposure or when improperly applied. If the prophylactic has not been applied at all or after four hours, the abortive treatment is indicated when it is practically certain that the sexual partner has acute open lesions of syphilis.

(2) Exposure of either sex to chronic, latent cases of syphilis with no open lesions, in whom the only evidence of syphilis may be a positive Wassermann reaction, does not call for abortive treatment even though prophylaxis has not been practiced. Under these conditions the chances of infection are so much less that I believe it is far better for the individual to take his or her chances and have the matter definitely settled by watching for the appearance of lesions to be followed by immediate, intensive treatment if such develop.

(3) The value of prophylaxis according to the method described in the preceding chapter has been more firmly established when properly applied within four hours after exposure, than has abortive treatment.

(4) Abortive treatment may be given any time from twenty-four hours to two weeks after exposure. But if more than two weeks have elapsed I believe it is better to wait for another week or two for the appearance of a chancre in order to settle the question, preparations being made to institute intensive treatment upon the first positive signs of syphilitic infection.

(5) The Wassermann reaction is of no value for detecting infection during this primary stage of syphilis. If it is done and is found positive, the individual is probably already syphilitic and almost surely will not develop a chancre even though repeatedly exposed. He is, however, a fit subject for antisyphilitic treatment.

Method of Abortive Treatment for Human Syphilis.—If abortive treatment is to be given, the results of animal experiments and clinical experience teach that it must be thorough to be worth while. Doubtless some cases may be cured by a single injection of arsphenamin or neoarsphenamin, a true example of Ehrlich's *therapia sterilisans magna*, but this is risky and not to be recommended even though treatment is given within twenty-four hours after exposure. Furthermore the administration of mercury and bismuth should not be considered at all; the best one may do is to administer the most active spirocheticides as arsphenamin, neoarsphenamin or sulpharsphenamin with particular care to chose the best brands of the latter on account of their variability in parasiticidal effects.

The doses and number of injections to be given vary according to the time elapsing since exposure; in my practice the following plan is employed:

(1) When exposure has been within three days: Two doses of arsphenamin (0.4 to 0.6 gm. each) at intervals of three days; or three doses of neoarsphenamin (0.6 to 0.9 gm. each) at intervals of three days; or three doses of sulpharsphenamin (0.4 gm. each) at intervals of three days.

(2) When exposure has been within four to seven days: Three doses of arsphenamin (0.4 to 0.6 gm. each) at intervals of four or five days; or four doses of neoarsphenamin (0.6 to 0.9 gm. each) at intervals of three to four days; or four doses of sulpharsphenamin (0.4 gm. each) at the same intervals.

(3) When exposure has been within eight to fourteen days: Four doses of arsphenamin (0.4 to 0.6 gm. each) at intervals of five days; or six doses of neoarsphenamin (0.6 to 0.9 gm. each) at intervals of three to four days; or six doses of sulpharsphenamin (0.4 gm. each).

(4) For periods longer than fourteen days I believe abortive treatment is inadvisable but if insisted upon, I recommend the same treatment as advised in the succeeding chapter for Wassermann negative cases of primary syphilis.

(5) The patient is kept under close observation with periodical thorough examinations of the parts and lymphatic system for at least three months. The Wassermann test may be done if expense is no particular consideration, but the reactions are usually negative since the Wassermann antibody or "reagin" is not produced in sufficient amounts for detection until the spirochetes have proliferated to the extent of producing tissue changes.

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CHAPTER LXXII

SPECIFIC TREATMENT OF EARLY SYPHILIS (PRIMARY AND SECONDARY STAGES)

Necessity for Early Diagnosis in Relation to Specific Treatment.—Since it is now easily possible in the majority of cases to establish the exact nature of the primary sore of syphilis by microscopical examination of the secretions for *Sp. pallida* and especially by the dark-field method, it is not necessary to follow the old custom of permitting the disease to progress to the stage of the cutaneous and mucous membrane eruptions (secondaries) before the diagnosis is made and treatment instituted. The Wassermann test is not of much value at this time, because the chancre must be present for about ten days before sufficient "reagin" or "Wassermann antibody" has been produced in the blood to give a positive reaction; indeed, a special effort should be made to establish the diagnosis and institute treatment before the Wassermann reaction becomes positive and to prevent it from ever becoming positive. As stated in Chapter XXXIX, the Wassermann test conducted with chancre secretions may yield a positive reaction, but as a general rule the dark-field microscopical examination is a better means for early diagnosis.

A large and constantly increasing experience and literature from all parts of the world testifies to the fact that the earlier treatment is instituted, the better are the chances for complete recovery; it is not improbable that many paretics and tabetics owe their condition to a failure of diagnosis on account of a small and atypical chancre and poorly-developed and evanescent secondaries which resulted in a failure of clinical diagnosis of syphilis and the institution of vigorous treatment during the early stages when most was possible from the therapeutic standpoint. It is true that as the disease is allowed to progress untreated, certain immunological changes occur in the chancre and each succeeding lesion tending to hinder progressive involvement of organs, but, as stated in Chapter XXXVII, the value and importance of these in relation to complete recovery from syphilis must not be over-rated and there is little or no evidence to indicate that human beings completely recover from syphilis unaided by specific medication. In other words, we are not justified in delaying the institution of specific medication in early syphilis simply to acquire the benefit of these immunological changes; our duty is to establish the diagnosis as soon as possible and to institute appropriate specific treatment without delay.

Classification of the Lesions of Early Syphilis in Relation to Specific Treatment.—As a general rule, the *chancre* or primary syphilitic lesion develops in two to four weeks after infection. The duration of this period of *primary incubation* varies according to the virulence of the infecting spirochetes, the numbers inoculated and the degree of tissue injury; but reported instances of incubation periods as brief as ten days and even one or two days are probably in error, infection having occurred at an earlier date than ascertained. Within a few days after the appearance of the chancre, the spirochetes have already invaded the lymphatic channels and neighboring lymphatic glands. No other changes may be apparent except the chancre, adenopathy and a peculiar characteristic pallor or anemia.

This constitutes the primary stage of syphilis according to Ricord's classification. As previously stated the Wassermann test does not usually yield a positive reaction earlier than ten to twelve days after the first appearance of the chancre and autoinoculability of the chancre usually terminates by the twelfth day; this period is designated by some German writers as the *prehumoral stage.* I do not like the term, however, because so much evidence indicates that spirochetes may be in the "humors" (blood and lymph) at this time and it is highly probable that minute amounts of the complement-fixing "reagin" or "Wassermann antibody" are likewise present, since Klauder and I have found it in the chancre secretions; we may not detect it in the blood simply because our methods are not sufficiently sensitive. However, from the standpoint of specific treatment it is serviceable to divide the chancre or primary stage into the *Wassermann negative* phase, during which autoinoculation is possible and may occur and the *Wassermann positive* phase, subsequent to which autoinoculation does not occur. As stated above a special effort should be made to establish the diagnosis and institute specific treatment in the former phase.

In the absence of treatment, the chancre slowly disappears and usually within six to seven weeks after its appearance, the skin and mucous membrane lesions appear associated with generalized adenopathy, the falling out of the hair, anemia and various symptoms constituting the *secondary stage* according to Ricord. In exceptional instances it is claimed that these lesions may develop any time within two years after the appearance of the chancre, but, from the standpoint of relation to specific treatment, I believe that the secondary stage of syphilis is to be classed with acute, early syphilis only when these lesions present themselves within two to four months after the appearance of the chancre; after this time the disease has actually entered upon its chronic stages since sufficient time has elapsed for fibrosis of capillaries and the early organization of lesions and the eradication of spirochetes becomes much more difficult with the demand for much more specific medication over longer periods of time. In other words, the acute skin and mucous lesions sometimes developing one or more years after the chancre are not secondaries in the usual meaning of the term, but simply acute exacerbation of chronic syphilis in which large numbers of spirochetes have been suddenly distributed to the skin with the production of similar lesions. The treatment of such, however, is essentially the problem of treatment of the underlying chronic lesions wherever they may be situated.

Acute, early syphilis therefore in my opinion, covers the first three or four months of the disease from the first appearance of the chancre or about five to six months from the approximate time of infection; when the disease is present for longer periods, I believe it is proper to designate it as "chronic" both from the standpoint of pathological tissue changes and specific therapy, even though the lesions may be so small or so situated as to fail to produce symptoms during the ensuing so called period of *latency or secondary incubation,* which is of variable duration, before detectable lesions and symptoms of the *tertiary stage* develop.

Advice to the Patient.—It is imperative for the physician to explain carefully to the patient the nature of the disease, its seriousness, its infectiousness and the necessity for guarding against spreading the disease and for thorough and prolonged treatment; also such directions as to diet, the use of alcohol and tobacco, the care of the mouth and general hygiene covered in Chapter LXIX.

It is quite common to find among the laity an exaggerated opinion of the curative activity of a few "shots" of "606." It is true that the adminis-

tration of arsphenamin or one of its substitutes removes the spirochetes from the open lesions much more rapidly than in the pre-salvarsan days, but the necessity for prolonged treatment and observation is scarcely less now than formerly; while I am opposed to gloomy prognoses and any statement tending unduly to frighten the patient, yet I believe it is well for the physician at the outset to impress upon the patient the fact that there is no real "short cut" to cure and that while complete cure is both quite possible and probable, it will be the reward only of careful and persistent effort and coöperation on the part of patient and physician. The responsibility of explaining the infectiousness of the disease and its danger to others by direct contact or parental transmission, as well as the necessity for treatment even after the obvious lesions have been dissipated, is especially onerous in public clinics, because so many of the poorly educated, and particularly colored men and women, are difficult to convince that treatment may be required even after lesions have disappeared and they are feeling and appearing in apparent good health. Printed slips are frequently useful for giving the necessary information and some scheme of this kind is always advisable when it is impossible for the physician to devote a sufficient amount of his own time and efforts in this very important part of the treatment of acute, early cases of syphilis.

Situation of the Chancre in Relation to Treatment.—Ordinarily, the situation of the chancre has no direct bearing upon specific treatment; that is, a chancre of the lip, for example, receives the same treatment as one on the genital organs. This is true, however, only when fairly intensive methods of specific therapy are employed; otherwise distinctions should be made. For example, the progress of a chancre of the lip should be promptly checked to prevent scarring as much as possible; furthermore, owing to rich vascular and lymphatic channels, systemic dissemination of spirochetes is probably greater than occurs in genital chancres. Wherever chancres are so located that ulceration may result in deformity or interference with functions, energetic specific therapy should be instituted as quickly as possible; while some syphilologists have stated that extragenital chancres are more refractory and systemic infection more likely than in the case of genital chancres, this has not been my own experience. I have seen chancres of the fingers, lip, tonsils, eye-lids, nasal mucosa, forehead, etc., yield as promptly and satisfactorily to specific medication as chancres of the genital organs.

Local Treatment of the Chancre.—This subject has received a great deal of attention and discussion in former years when it was commonly believed that syphilis was purely a localized infection during the primary or chancre stage. Hunter, Ricord and even more modern syphilologists have been warm advocates of excision or cauterization of the chancre with the idea that such measures effectually removed the disease. Circumcision and cauterization are still practiced for this purpose and not infrequently we meet with chronic cases of syphilis to-day among patients who were assured by their physicians that cauterization of the sore with carbolic acid, silver nitrate, etc., had removed all danger. Such measures applied to non-syphilitic lesions as herpes, chancroids or simple abrasions mistaken for syphilis, may readily enough lead both physician and patient into the mistaken conclusion that local treatment resulted in a complete cure and removal of syphilitic infection, but there is now available overwhelming clinical and experimental data showing that circumcision and cauterization of a true syphilitic chancre rarely succeeds in removing all infection and subsequent generalization of the disease.

Histological examination of excised penile chancres shows that the lymphatic spaces are invaded by spirochetes and lymphocytes for an area of

almost an inch or more beyond the lesion, even though these changes are detectable only by microscopical examination; it may be that a chancre located on the tip of a long foreskin and excised during the first day or two with a wide sweep of apparently uninfected tissue may be effective, but these are certainly exceptional and even rare circumstances. Furthermore it would appear that spirochetes may be present in the inguinal glands within a few days after the chancre appears; even fifty years ago this fact was appreciated and some went so far as to advise injections of mercury to block off the lymphatics between the chancre and the glands and even to excise the latter, but in view of the large number of lymphatics and glands and since the glands in both groins are involved, these suggestions now appear foolish and only of historical interest.

If, however, a chancre occurs on a foreskin long enough to produce phimosis, therefore rendering circumcision advisable for general reasons, or upon the labia minora, rendering easy the matter of excision, the operation of removal may be conducted and is frequently recommended by modern syphilologists, but never with the advice to the patient that the operation removes the disease, since the incidence of failures has far exceeded the apparent successes; at least, an enormous number of spirochetes are removed *en bloc*, but another chancre may develop in the scar and an important depot for antibody production is removed, rendering very hazardous any dependence alone upon excision for the cure of syphilis. Indeed, this is so true that the excision of a true syphilitic sore (chancre) should always be followed by thorough and vigorous specific medication, since it is entirely likely that the spirochetes escaping removal will now be able to proliferate and migrate even more vigorously and extensively than would otherwise be the case.

If circumcision, therefore, is so likely to fail that it occupies no place in the treatment of syphilis insofar as dependence is to be placed upon it alone for eradication of the infection, how much more likely is cauterization to fail! And yet not a few physicians and the laity believe in its efficacy. A host of substances have been advocated and especially phenol followed by alcohol, sulphuric acid, fuming nitric acid, silver nitrate, copper sulphate, the actual cautery, etc. All of these produce immediate coagulation necrosis of the tissues and, while it is freely admitted that those spirochetes directly reached may be destroyed, how about those which may not be reached in the deeper portions of the chancre and in the margin of the lesion? It is foolish to even expect their destruction by such means, not to mention the pain produced and resulting necrosis which may become secondarily infected with pyogenic bacteria with the production of phagedenic ulcers and suppurative adenitis. Circumcision may actually accomplish something worth while when followed by adequate specific treatment; there is nothing to be said in favor of cauterization of the chancre insofar as the cure of syphilis is concerned. Sometimes, however, the chancre requires vigorous local treatment and especially if phagedenic; for this purpose the treatment proposed by Bausbach[1] is to be recommended, consisting of calomel 10 parts, zinc sulphate 10 parts, and chlorin water 50 parts applied on a piece of cotton, covered with oiled silk, and left in place for twenty-four hours.

As a general rule, the chancre should be merely treated with wet dressings of saline solution until the diagnosis has been made preferably by microscopical examination of the secretions for *Sp. pallida* and especially by the dark-field method. Dressings of this kind are usually sufficient for promoting drainage, particularly if hypertonic saline (10-per cent.) is employed, and for keeping the sore free of excessive suppuration. After the diagnosis has been established the sore may be treated with 1:1000 bichlorid of mercury

preferably in 10-per cent. saline solution, boric acid solutions, 33-per cent. calomel ointment or by dusting powders of calomel, aristol, etc. The wet dressings, however, are preferred when there is considerable mixed infection and suppuration with adenitis, because they do not hinder absorption of the pus into the dressings as may an ointment or the heavy application of a dusting powder. As a general rule, I prescribe the bichlorid dressing for the first week, followed by the ointment and lastly by a dusting powder until the sore has completely healed. A dusting powder of arsphenamin may be employed, but it is more expensive, is apt to discolor the underclothing and is probably not any more spirocheticidal than calomel.

Choice of Drugs for the Specific Treatment of Primary and Secondary Syphilis.—Since the indications are for the destruction of spirochetes as rapidly and thoroughly as possible, practically all syphilologists at the present time are agreed that arsphenamin, neoarsphenamin or sulpharsphenamin are the remedies of choice; when for any reason these drugs cannot be given, probably bismuth ranks next and mercury third. The iodids are so feeble in spirocheticidal activity that they are not to be considered at all as primary remedies in acute, early syphilis, although they may be given later with advantage. The soluble and insoluble salts of mercury in ordinary doses are almost without effect upon the spirochetes in the chancre and mucous patches although these lesions gradually heal under mercurialization; even large doses approaching toxic effects influence the spirochetes much less promptly than arsphenamin and its substitutes, as shown by Scholtz and Kelch,[2] Ineyrat[3] and others.

However, mercury still has a definite place in the treatment of acute, early syphilis. While there are numerous cases on record of apparent cures from arsphenamin or neoarsphenamin alone, yet to-day there is a growing tendency in favor of mixed treatment and my own practice is to administer courses of mercury after arsphenamin or one of its substitutes. This is primarily as a safeguard for the destruction of those parasites entrenched in the lymphatic glands or other tissues and escaping arsenical destruction.

The matter of choice of arsenical is largely one of individual opinion. Since 1918 neoarsphenamin has become undoubtedly more popular than arsphenamin, largely because of its greater ease of administration and greater freedom from toxic reactions permitting more intensive treatment than is usually possible with arsphenamin. I believe that neoarsphenamin is to be preferred for these reasons, but only when a neoarsphenamin of superior parasiticidal properties is employed. As I have discussed with more detail in Chapter LXVII, to use neoarsphenamin merely on the basis of greater ease of administration and low toxicity for rats is a mistake; it is true that most all compounds including the much less spirocheticidal pentavalent compounds, produce healing of the lesions as this is not a difficult task, but when reliance is placed upon neoarsphenamin in this critical period of therapeutic opportunity, due care must be taken to build with a foundation of rock rather than with one of shifting sands. With arsphenamin there is much less opportunity for an error of this kind; undoubtedly it is the remedy of choice at this stage of the disease when the best spirocheticidal effects are desired because it is the most uniform and active spirocheticidal agent available at the present time.

Tzanck and Dohen[4] have employed sulfarsenol by subcutaneous injection but on account of numerous failures have expressed a preference for large doses of neoarsphenamin by intravenous injection; Stokes and Behn,[1] however, have recently stated that intramuscular injections of an American brand of sulpharsphenamin have yielded them as good clinical and serological

results (92.8 per cent. cures) in primary and secondary syphilis as in patients treated previously in a similar manner with arsphenamin (94 per cent. cures). Certainly when intravenous injections of arsphenamin and neoarsphenamin cannot be given, intramuscular injections of sulpharsphenamin should be resorted to, the method employed by Stokes and Behn being given later in this chapter.

In reference to the choice of a mercurial compound, I believe that inunctions are to be preferred when it is possible to apply them in a thorough and acceptable manner; otherwise, intramuscular injections should be given, my preference being for the succinimid in almond oil with the salicylate in oil as second choice. When, however, it is necessary to rely mostly upon mercury for the treatment of those exceptional cases of hypersensitiveness to arsenic, I believe that intravenous injections of flumerin or mercurosal are first choice for a short series of administrations followed by intramuscular injections of the succinimid; mercury by ingestion should never be resorted to for the treatment of primary and secondary syphilis when the most prompt spirocheticidal effects are indicated and desired.

Influence of Specific Medication upon Immunity in Early Syphilis and the Dissemination of Infection, Especially in Relation to Neurosyphilis.—Does arsphenamin and its substitutes employed in the treatment of early syphilis increase the incidence of neurosyphilis? There are some physicians who believe that the incidence of neurosyphilis, cardiovascular syphilis and chronic syphilitic infections of other organs has increased since the advent of salvarsan and that the onset of neurosyphilis has been precipitated or hastened in some cases by the use of this drug. Probably this impression is due in part to our improved methods for the clinical and serological diagnosis of syphilis and especially of neurosyphilis by examinaton of the cerebrospinal fluid, but aside from this it is pertinent and important to examine the question in an unbiased manner.

As explained in Chapter XXXVII, it has been quite definitely proven that when early syphilis is left alone and untreated, the tissues are capable of setting up some immunological mechanism of cellular resistance to the parasites and some physicians believe that in early syphilis we may do well to revert back to the practice of older syphilologists in delaying treatment until the secondaries appear, not so much to clinch the diagnosis as was their purpose, but to give the body sufficient time to muster or mobilize its cellular resistance. Now this cellular resistance is stimulated only by living spirochetes; vaccines of dead spirochetes unfortunately do not aid, as explained in Chapter XXIII. The only way to obtain it at present is by leaving sufficient living spirochetes in the tissues. If we reduce their numbers by sub-sterilizing doses of spirocheticides, the results of animal experiments have shown that the survivors meet with less resistance, are more quickly disseminated and more quickly produce infections of distant organs. It is conceivable that the specific treatment of early human syphilis may have the same effects and similar results and that our best spirocheticides, like arsphenamin and its substitutes, may actually spread the parasites in the body if given in amounts only sufficient for dissipating the chancre and adenopathy but short of complete sterilization. On the other hand, if we leave the cases untreated dissemination will occur anyhow, although not so rapidly, and there is practically no hope that cellular resistance alone will extirpate the infection or hold the disease in indefinite latency. So there we are—we must make our choice.

What should we do? Deliberately wait until the organisms are widely disseminated and the secondaries produced before treatment is instituted in order to obtain the maximum degrees of cellular resistance and especially

from the skin, which organ is apparently particularly active in this regard? Or treat with mercury by ingestion or injection after Fournier's plan which served to hasten the healing of the chancre and to mitigate the secondaries but probably leaving enough surviving spirochetes to stimulate cellular resistance? Or try to obtain similar results with small doses of arsphenamin and its substitutes which however is much more difficult to accomplish because if the dose is too small the spirochetes may be actually stimulated? Or should we endeavor to affect a radical cure by the prompt and vigorous administration of arsphenamin and its substitutes realizing that we may occasionally fail but that success is apparently in reach in the majority of cases because the vital organs are in such good general condition that we need not greatly fear dangerous toxic or organotropic effects?

The last is my choice for the treatment of the majority of cases of early syphilis in good general condition, because present results indicate that the incidence of chronic syphilis developing among intensively treated early cases is less than 5 per cent. I realize, however, that common sense must be exercised in dosage. Too much arsphenamin or neoarsphenamin may do harm if toxic tissue changes and capillary injuries are produced. I believe that it is possible to effect radical cures, however, without discernible damage; at least, the damages are apparently rapidly healed. On the other hand, I believe it is to be admitted that insufficient arsenical therapy may actually do harm in the way of depressing cellular resistance by sub-spirocheticidal effects, enabling surviving organisms to produce early lesions in the central nervous system and other distant organs. Since the general practice is to give too little arsphenamin and neoarsphenamin, usually enough to dissipate the lesions but short of complete sterilization, it may be indeed true that the incidence of neurosyphilis has actually increased during the past fifteen years. I believe that the old fashioned chronic intermittent mercury treatment is to be preferred to a few desultory injections of arsphenamin or neoarsphenamin, but that best results are secured by bold and thorough arsenical treatment within common sense limits; the latter is my practice and the method of application shortly follows.

Dosage and Intervals of Administration of Arsphenamin and Its Substitutes in the Treatment of Primary and Secondary Syphilis.—One of the results of the discovery of arsphenamin and neoarsphenamin was to foster the long cherished hope for a standardized method for the treatment of syphilis, at least for early syphilis in the primary and secondary stages of the disease. The dose to employ, the number of injections to be given, the intervals between injections, the number of injections constituting a course and the number of courses to be given have ever been perplexing subjects calling forth a voluminous literature detailing various methods and plans. If we had a sure and applicable method for determining when a syphilitic is really cured in the sense of complete eradication of all spirochetes, analogous, for instance, to the gland transplants of rabbits, the problem would be much easier to solve; but in human syphilis we can rely only upon the dissipation of obvious lesions, negative complement fixation reactions with blood serum, negative results of cerebrospinal fluid examinations and, lastly, but most important of all, upon the final clinical records of individual patients during the balance of their lives. We do not know therefore in accurate terms, what may be the real value and what will be the final outcome, of our modern plans of treatment. At present we must rely upon rigid clinical examinations and especially for neuro-recurrences, repeated blood complement-fixation tests with the most sensitive but specific methods and examinations of spinal fluid, as our guides and criteria while waiting for Time to close

the chapter. It is extremely difficult to evaluate present day methods of treatment because of wide variations in the clinical skill and judgment of those reporting their results and especially because of a deplorable lack of sensitiveness and accuracy of serological tests upon which opinions are based.

How can the radical or complete cure of syphilis in the primary and secondary stages be best attained? We must seek for our answer in the clinical experiences of others and ourselves employing different preparations, varying doses and varying plans of administration; also from the results of carefully planned therapeutic experiments with the lower animals infected with syphilis and finally base an opinion upon these with the reservation that it may require revision in the light of future experience as we are able to follow our patients through the balance of their lives.

Keeping in mind the main facts concerning the rates of absorption and elimination of arsphenamin and its substitutes, the probable mechanism of their spirocheticidal activity, toxicity for body cells and ultimate fate as discussed in previous chapters, what constitutes the best plan for their administration? Is a large dose at long intervals, say seven days' as good or better than a large dose at close intervals? Is a small dose at close intervals as good or superior? How much must be the total administered by either plan for radical effects? As pointed out by Hatcher and Wilbur,[6] the intensity of the action of a given drug usually depends largely on the total amount that acts at one time, and this is determined to some extent by the ratio between the rate of absorption, on the one hand, and that of elimination or destruction in the body, on the other. A large dose of a drug may produce less effect at one time than a small dose administered in the same way at another time, the difference in the intensity of action being due to differences in the rates of absorption and elimination. It is not equally well understood, however, that low concentrations of a chemical compound may affect protoplasm in a distinctly opposite manner from that provoked by larger proportions; for example, a very small dose of arsphenamin or neoarsphenamin may actually prove dangerous from the standpoint of stimulating the multiplication and activities of spirochetes instead of destroying them, as shown by Bronfenbrenner and Schlesinger[7] in the treatment of syphilitic rabbits. Brock[8] states that doses of 0.03 to 0.04 gm. of arsphenamin for adults neither cures nor favorably influences acute early syphilis; Balzer,[9] on the other hand, states that the daily subcutaneous injection of sulfarsenol in dose of 0.010 to 0.030 gm. until a total of 4 to 5 gm. has been given yields excellent results. Scholtz and Kelch[10] state that a total of 0.2 gm. of arsphenamin divided into two doses a day of 0.05 gm. each for two days followed by two doses of 0.25 gm. on the third day and one dose of 0.3 gm. on the fourth day is the most effective method for bringing about a rapid disappearance of spirochetes from chancres.

A great deal of attention is being given at present to intensive treatment, improperly designated as "abortive treatment" by German and French authors. Pollitzer[11] in America and according to Artz,[12] Notthaff in Germany, have been pioneers in the method of "striking hard" by short intensive courses comprising three or more daily injections of 0.4 to 0.6 gm. of arsphenamin. Ineyrat and Pinard[13] have likewise subscribed to this plan beginning with 0.15 gm. of arsphenamin and reaching 0.6 gm. by the eighth injection. Katz[14] claimed many years ago that 0.3 gm. of arsphenamin as a dose was just as effective as the 0.6 gm. dose recommended by Ehrlich, and Hazen,[15] Ormsky,[16] Ballenger and Elder,[17] Goodman,[18] Gennerich,[19] Oliver,[20] Polland[21] and others, while advocates of intensive treatment are in favor of

small doses of arsphenamin (0.2 to 0.4 gm.) or neoarsphenamin (0.4 to 0.5 gm.) rather than the maximum doses of 0.6 gm. and 0.9 gm. respectively.

Pollitzer has advocated at least three daily doses of arsphenamin, but many others have found this plan inadvisable if toxic reactions are produced. I have likewise abandoned it when toxic reactions are produced, although it is usually possible to give three daily doses of 0.3 gm. arsphenamin (disodium salt) in 30 c.c. by syringe injection without untoward effects; within the last few years I have used neoarsphenamin in three to six daily doses in amounts of 0.6 gm. each with apparently equally good therapeutic results and remarkable freedom from untoward effects. The majority of syphilographers, however, have been more in favor of longer intervals between injections, as two per week of neoarsphenamin and one of arsphenamin every 5 to 7 days.

Whatever may be the dose and interval of injection employed, it would appear that most writers claim that at least a total of 4 to 5 grams of arsphenamin or neoarsphenamin are required for the cure of primary and secondary cases of syphilis. The old idea of Ehrlich of curing the disease with a single injection of 0.6 gm. of arsphenamin was soon disproven even in the treatment of the earliest chancres, although this dose may bring about the prompt disappearance of the spirochetes from the lesion followed by prompt healing. Biele[22] mentions a woman who received 3 gm. of neoarsphenamin in one injection in mistake for 0.3 gm. Her Wassermann reaction became negative without further treatment five weeks later although the patient had had a previous injection of 0.45 gm. of neoarsphenamin and some mercury. It is interesting to note that only slight toxic symptoms followed the large single dose; these disappeared within five days after injection.

Various Methods Proposed for the Treatment of Primary and Secondary Syphilis.—As previously stated, a very large number of plans and methods for the treatment of primary and secondary syphilis have been described in the literature and Baketel[23] has recently published many more from personal correspondence with American syphilologists some of which I am quoting below from his article; space does not permit me to present all of these, but some are given herewith as examples to show the trend of thought and practice in the search for a standard method:

Fordyce and Rosen, of New York, treat cases with positive dark-field and negative Wassermann reactions with 8 intravenous injections of arsphenamin in dose of 0.3 to 0.5 grams, the first three every other day, and the remaining five, every five to seven days. The second course of six injections is given after an interval of four to six weeks. Patients who have never developed positive serologic findings are considered as aborted cases and are under observation for at least a year, with monthly blood tests. If a positive Wassermann reaction develops during treatment, the patient is given the same treatment as the early secondary cases, namely, three injections of arsphenamin, one every other day of 0.3, 0.5 and 0.6 gms., and the remainder every five to seven days until eight injections have been given. Mercury is given during this course consisting of 15 intramuscular injections of salicylate, grs. 1 to 1.5, every seven days or, if soluble mercury is employed, bichloridol, gr. 1, every four or five days. Notwithstanding a possible negative result, the treatment is repeated after the patient has had a four to six weeks' rest, being given eight more weekly injections of arsphenamin and 15 of mercury. If necessary, the patient is given a third course of the same number of treatments after the usual period of rest. Should the Wassermann test be negative, a rest of three months is advised followed by a course of 6 arsenic and 10 mercury injections.

Schamberg, of Philadelphia, advises that in the treatment of primary syphilis in an individual in average good health and of normal weight, two doses of 0.9 gm. neoarsphenamin may be given each week for two to three weeks, followed by weekly injections until ten administrations in all have been given. This is provided the drug is well tolerated. After this, a course of mercurial injections or inunctions are given. At the end of this period if the Wassermann reaction is negative, the remainder of the treatment may be mercurial. Mercurial pills may be given over a period of some months to insure cure.

This treatment represents in a way a combination of the new with the old treatment of syphilis. In the event of a positive Wassermann, neoarsphenamin is again administered every week for twelve injections and again followed by mercurial injections or inunctions.

The treatment of secondary syphilis is largely that of primary syphilis save that more courses of treatment are given, independent of the outcome of the Wassermann test. In other words, there is an irreducible minimum of treatment. Three courses of 8 to 10 injections of the arsenical employed, with three courses of mercurial injections or inunctions constitutes such a minimum in his opinion. A negative Wassermann reaction after a brief course of treatment is by no means regarded as an indication of the death of all of the spirochetes in the body. His experience demonstrates that lapse of treatment at such times is commonly followed by a recrudescence of a positive reaction. No definite rule is set down as to the number of treatments which any given patient may require. After a negative Wassermann reaction, mercurial treatment by month is continued for long periods.

Moore, of Baltimore, begins the treatment of early syphilis with arsphenamin, the first course being of eight doses and succeeding courses of six doses each. Mercury by inunction is started just before the last dose of each arsphenamin course, and continued until the first dose of the next. The interval between arsphenamin courses is at first short (four weeks), and is gradually lengthened with each course. The longest interval permitted is twelve weeks. Spinal puncture is performed as a routine after the first or second course of arsphenamin. The treatment of Wassermann negative primary syphilis usually lasts exactly one year, and consists of at least four courses (26 doses) of arsphenamin and twenty-six weeks of mercury. In Wassermann positive primary and in secondary syphilis, the length of treatment in the average case is usually 15 months (five courses of arsphenamin and thirty-two weeks of mercury). If spinal fluid abnormalities are found at the time of the first puncture, this plan is modified according to the findings. When treatment has been completed, there ensues a year of probation, during which there must be no symptoms, no positive and at least six negative Wassermann tests (ice-box); and, at the end of the year, negative physical and neurologic examination, negative *x*-ray of the cardiovascular stripe, and negative examination of the spinal fluid. Moore is of the opinion that continuous treatment without periods of intermission insures in greatest degree against the possibility of relapses, and especially infection of the central nervous system. For this reason mercury is started before the course of arsphenamin injections is finished and continued to the first injection of the succeeding series of injections. Moore believes that if this plan of treatment could be applied to every case of primary and secondary syphilis, the incidence of asymptomatic neurosyphilis, and in all probability that of clinical neurosyphilis, might be reduced to an absolute minimum. Judging from his data the present level of 20 to 25 per cent. of clinical neurosyphilis might be reduced to 5 per cent. or less. The economic saving to the state, to mention only one of the advantages of this reduction, would be enormous.

Ormsby,[24] employs the following method: In the early stage, before generalization has occurred, three injections of arsphenamin are given with an interval of one day between the injections. The average dose is 0.4 gm., but in some instances 0.5 or 0.6 gm. is given if the patient is large and vigorous. In patients not previously treated the first dose is never more than 0.2 gm. Following this, one month's treatment with mercury, either by injection or inunction, is employed. Six weeks from the last arsphenamin injection, three more are given. In well-developed cases not previously treated, the same procedure is employed, except that after the second series of arsphenamin twelve injections of mercuric salicylate are given at weekly intervals, or their equivalent in inunctions over a period of three months, when a third series of arsphenamin is given as before, this being followed by another three months of mercury. In the second year two series of treatments are given in the Wassermann negative cases as a prophylactic measure. In the Wassermann positive cases the procedure is as outlined for the first year.

Stokes[25] begins treatment with three injections of arsphenamin at intervals of three days followed by five more at weekly intervals, followed by two to four more courses of six injections each at weekly intervals with periods of rest so that from 18 to 24 injections are given during the first year along with a total of 300 inunctions in courses of 40 and 80. In the treatment of early syphilis with sulpharsphenamin by intramuscular or epifascial injection (0.2 gm. in each buttock), Stokes and Behn gave three injections at three day intervals, followed by one injection every five days until eight injections had been given. Following the third injection, inunctions of mercury are begun and continued in courses of 80, bridging the sulpharsphenamin intervals. The second, third and fourth sulpharsphenamin courses follow one another at intervals of six weeks.

Gennerich[26] administers arsphenamin by intravenous injection in dose of 0.2 to 0.5 gm. for men and 0.1 to 0.3 gm. for women, every three to four days for the first four doses and then at intervals of five to seven days for an additional four to six doses. Mercury by inunction or an insoluble salt by intramuscular injection is given for six weeks. Gennerich is very much opposed to the administration of neoarsphenamin in primary and secondary

syphilis and administers small doses of arsphenamin. In the treatment of syphilis in the secondary stage treatment is the same followed by five weeks' rest and four to six more injections of arsphenamin; mercury is not given. If the disease is of more than three months duration another course of six injections is given followed still further by a final course accompanied by the administration of mercury.

Finally mention should be made of the observation of Greene[27] who claims that after syphilis is brought under control, arsphenamin should be administered at twenty-eight day intervals on the basis of theoretical considerations indicating that *Sp. pallida* has a life cycle in which it is resistant during a resting stage of encystment and vulnerable every twenty-eight days as it passes into a more active form. Thomson[28] has advocated a somewhat similar plan consisting of the intravenous injection of neoarsphenamin every three weeks continuously until the Wassermann test yields a negative reaction at which time nercury with chalk is given by ingestion for at least a year.

These various quotations from the literature showing the methods employed by syphilologists in different parts of the world on the treatment of acute, early syphilis in the primary and secondary stages will at least indicate how divergent are the methods employed in America and Europe; indeed, every syphilologist appears to have a method more or less his own. They also show a tendency toward thorough treatment and that dependence is seldom placed upon arsphenamin alone or its substitutes. The subject appears almost in a chaotic state, but such is not the case for those possessing a good working knowledge of the pathology and immunity of syphilis and the pharmacology, toxicology and therapeutic applications and limitations of the drugs employed. With such the treatment may be arranged for each case according to the patient's individual condition, the chief demands being intensive and intelligent treatment of each case during these early stages when the most favorable opportunity is offered for the best therapeutic results. Since there are so many methods advocated it would appear almost superfluous to add others, but, as just stated, everyone seems to have their own and so do I, given here for what help they may be to others.

Author's Method for the Treatment of Syphilis in the Chancre or Primary Stage.—If primary syphilis of human beings were as easily cured as primary scrotal syphilis of rabbits this result could be obtained by a single intravenous injection of about 0.9 gm. arsphenamin (1.5 gm. neoarsphenamin) per 145 pounds; or by a total of 1.3 gm. arsphenamin (1.7 gm. neoarsphenamin) divided into 3 daily doses; or by a total of 2.1 gm. arsphenamin (3.2 gm. neoarsphenamin) divided into 6 doses at three day intervals; or by a total of 2.1 gm. arsphenamin (4.2 gm. neoarsphenamin) divided into 6 injections at weekly intervals. But it is evident that this is not the case and no one can state how much more per kilogram of weight is required by human beings for the complete cure of syphilis in the early chancre stage, but it is a safe guess that at least two to three times as much are required.

From the standpoint of the treatment of primary syphilis, it is helpful to divide the cases into those diagnosed and treatment instituted in the first ten days after the appearance of the lesion when the Wassermann test is negative and the lesion autoinoculable and those after this period, when the Wassermann test is apt to be positive and the lesion less likely to be autoinoculable; the only difference, however, is that the latter type may require more thorough treatment than the former although I usually treat both groups alike on the as sumption that a little more treatment than may actually be required for the former will do no harm. My plan of treatment is as follows for adults of both sexes with genital or extragenital chancres and accompanying lesions and symptoms:

1. A physical examination is made, the urine examined, the patient advised regarding the nature of the infection and given the hygienic directions described in Chapter LXIX with special reference to the care of the mouth.

2. The lesion is treated locally as described on page 947.

3. Three daily successive intravenous injections are given of neoarsphenamin in dose of 0.6 gm. or of arsphenamin in dose of 0.3 gm., the latter being of course in alkaline solution (disodium salt) and sometimes administered in a volume of 30 c.c. with a syringe. The injections are given at least two hours after a preceding light meal and constipation is relieved beforehand.

4. These injections are followed by six more of neoarsphenamin in dose of 0.9 gm. or arsphenamin in dose of 0.4 to 0.6 gm. (the latter if well borne) at the rate of two injections per week.

5. I have not employed sulpharsphenamin, but in cases to whom intravenous injections cannot be given, the plan of Stokes and Behn may be followed: three epifascial injections of 0.4 gm. (0.2 gm. in each buttock) at three-day intervals, followed by an injection every five days until eight injections have been given.

6. Immediately after the last dose of neoarsphenamin, arsphenamin or sulpharsphenamin, mercury by daily inunction is started and maintained for six weeks (42 inunctions). The dose is one dram of 50 per cent. mercurial ointment or 85-per cent. calomel ointment applied as described in Chapter LXIII. If for any reason inunctions cannot be given or if it becomes apparent that they are not being applied regularly and faithfully (as is not infrequently the case), intramuscular or epifascial injections of potassium tartrobismuthate in dose of 0.2 gm. in a vegetable oil or 1 to 1½ grains of mercury succinimid or mercury salicylate in oil are given once a week for six weeks; the tartrobismuthate, however, is much less painful and is always preferred especially for sensitive individuals. The urine is examined every two weeks with weekly inspections of the patient for evidences of mercurialism or bismuth siderosis, etc.

7. This is followed by a rest of four weeks with mild specific medication in the form of protiodid (¼ grain three times a day) or 1 grain of mercury with chalk together with 1 grain of Dover's powder three times a day. Intermissions without specific treatment is considered unwise in order to prevent relapses and infection of the central nervous system. A Wassermann test is then conducted, but, regardless of whether the reaction is positive or negative, treatment is continued. A negative reaction, however, gives a more favorable prognosis.

8. Eight more intravenous injections of neoarsphenamin in dose of 0.9 gm. are now given at intervals of five to seven days; or six intravenous injections of 0.3 to 0.4 gm. arsphenamin at weekly intervals or eight epifascial injections of sulpharsphenamin in dose of 0.4 gm. at the same interval.

9. Beginning with the last dose of arsenical the same course of bismuth or mercury (inunctions preferred) described above is repeated followed by a rest of six weeks with mild mercurial medication by ingestion, when a Wassermann test is made. If treatment had begun in the first ten days of the chancre before the Wassermann test yielded a positive reaction and if the two tests called for in this plan have yielded negative reactions, it is permissible to stop the injections, but to examine the spinal fluid and continue with the treatment described in paragraphs 12 and 14. If, however, treatment was started later when the Wassermann reaction was positive, it is well to continue as follows:

10. Six more intravenous injections of neoarsphenamin in dose of 0.6 to 0.9 gm. every seven days; or four intravenous injections of arsphenamin in dose of 0.3 to 0.4 gm. every seven days or six epifascial injections of sulpharsphenamin may be given in dose of 0.4 gm. at the same intervals.

11. Beginning with the last injection of arsenical, mercury is administered again for six weeks by inunction or injection; bismuth is not given at this time to make sure that the patient receives at least one course of mercury.

12. This is followed by a Wassermann reaction on the blood and an examination of the cerebrospinal fluid for total cells, protein, Wassermann and colloidal gold reactions. If positive findings are observed the balance of treatment must take into consideration the early cerebrospinal infection as described in Chapter LXXVI.

13. Up to this point the patient has received either 23 intravenous injections of neoarsphenamin, a total of 11.7 gms. if each dose has been 0.9 gm.; or 19 intravenous injections of arsphenamin, a total of 7.6 gms. if the dose has been 0.4 gm. (as is usual in my practice); or 24 injections of sulpharsphenamin, a total of 9.6 gms. if the dose has been 0.4 gm. each as advised by Stokes and Behn. At the same time either 126 drams of mercury by inunction or 18 epifascial injections of mercury (18 grains) have been given or 12 epifascial injections of bismuth (2.4 gms.) and 6 of mercury (6 grains) in addition to the mercury taken by ingestion.

14. If the blood serum and spinal fluid tests have yielded negative reactions along with the total absence of all clinical manifestations of syphilis, the patient should be kept under observation for at least one year from this time taking either 1 grain of protiodid of mercury or 3 grains of mercury with chalk per day in divided doses for periods of two months followed by one month of rest and a blood Wassermann test at the end; this means therefore eight months of mercury by ingestion with four Wassermann tests during this period.

If, however, the spinal fluid tests yield positive reactions or, if at any time during the year of probation, the blood Wassermann test yields even a weakly positive reaction, it is my custom to give another course of arsenical injections followed by bismuth or mercury as described in paragraphs 8 and 9 or after the plan described in Chapter LXXVI for the treatment of early neurosyphilis.

Author's Method for the Treatment of Syphilis in the Secondary Stage.—The same plan is pursued as described above for the treatment of syphilis in the primary stage except that it is more prolonged and the iodids are included, because of the wider dissemination of the spirochetes through the body and their longer residence on the tissues with the production of tissue changes. For these reasons the possibility of early neurosyphilis with spinal fluid changes must not be overlooked and it is especially necessary to keep these in mind in any plan or method for the treatment of syphilis in the secondary stage. No case is dismissed without at least one spinal puncture toward the end of a treatment unless this is absolutely refused by the patient; to do otherwise may be a serious error.

1. Treatment is given in succession as described in paragraphs 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 for the treatment of primary syphilis. It is continued with mercury as described in paragraph 11 except that potassium iodid is given in addition by ingestion over the period of six weeks in dose of at least 30 grains per day for women and 45 for men in divided doses as described in Chapter LXV; if not well tolerated, smaller doses are given.

2. This is followed by examinations of the blood and spinal fluid as described in paragraph 12. If all tests yield negative results, one may continue as described in paragraph 14, but my usual practice is to treat as follows during this year of observation:

3. Two months of mercury and potassium iodid by mouth followed at once by four weekly injections of neoarsphenamin in dose of 0.9 gm., or arsphena-

min in dose of 0.4 gm. This is followed by one month of iodid by mouth and mercury by inunction or four weekly injections of potassium tartrobismuthate in dose of 0.2 gm. each or four weekly injections of mercury succinimid or salicylate in dose of 1 grain each.

4. This is followed by two months of rest without specific medication when a Wassermann blood test is made.

5. The course described in paragraph 3 is now repeated even though this last blood reaction is negative.

6. Two months of rest are now given followed by a blood Wassermann test and a second (final) examination of the cerebrospinal fluid. If the latter shows an increase of cells, protein and positive Wassermann and colloidal gold changes, treatment is modified as described in Chapter LXXVI for early neurosyphilis. No case is over discharged without examination of the spinal fluid unless this is absolutely refused by the patient.

Standardization of Specific Treatment of Early Syphilis.—The methods just given are applicable in my experience for the specific treatment of the majority of cases of early syphilis of both sexes infected genitally and extragenitally. They are, however, intensive and may be objected to on the basis of being unnecessarily thorough. I can only state that it is my conviction that early syphilis requires some such thorough treatment if complete and lasting serological as well as clinical cure is to be obtained. It is freely admitted that much less treatment usually suffices for dissipating the lesions and restoring the patient to a state when he or she feel and appear perfectly well, but Wassermann tests conducted by a *sensitive* method may yield positive reactions again within a year or two after cessation of treatment and, in my opinion, this regrettable finding is an index of continued infection and an indication for the resumption of treatment. I would wish to emphasize with all the force at my command that a truly positive Wassermann reaction under the circumstances indicates infection and that the test should be conducted with a method as sensitive as possible with specificity to insure sufficient treatment. The methods of treatment given above are based upon the demands of my new quantitative complement fixation method; less, much less, treatment may result in dissipating the infection to such a degree that less sensitive methods will yield negative reactions, but they are apt to be falsely negative and misleading because insufficient for detecting slight evidences of residual spirochetic infection.

In the usual and average case of early syphilis we can truly treat the disease and largely ignore the patient, because most infections occur in young and otherwise healthy individuals; this is in sharp contrast with the treatment of chronic syphilis when just the reverse usually pertains. But not all cases can be treated alike. Some are intolerant of arsphenamin and its substitutes, requiring smaller doses and longer intervals than usual or indeed, the entire abandonment of arsenical therapy. In others tuberculosis, cardiovascular disease, chronic nephritis, infections of the liver and gall-tracts, etc., may introduce complicating factors demanding a careful choice of medicaments and methods of administration to avoid treating syphilis to the detriment of the patient. It is these exceptions that render difficult and unwise any attempt toward formulating fixed or standard methods of treatment of early syphilis but the problem is infinitely less complicated than attempts of this sort in the treatment of chronic syphilis. The treatment of these exceptional and difficult cases of early syphilis is, however, too important a subject to be briefly dismissed and the subject is discussed with more detail in Chapter LXXVIII.

Minimum of Treatment for Early Syphilis.—It is ordinarily so easy to dissipate the early lesions of syphilis like the chancre, the skin and mucous membrane and associated lesions of the secondary stage that great care must be exercised against concluding that the disappearance of these means that a complete and radical cure of the disease has been accomplished. If in addition the Wassermann tests are conducted by a method lacking in sufficient sensitiveness the danger of under treatment is greatly intensified. Less, much less, treatment than advised in the foregoing sections will cause the disappearance of the lesions and yield negative Wassermann reactions when the tests are conducted according to the original method and especially if plain alcoholic extracts of syphilitic liver or other tissue are employed as antigens. It is far better not to use the Wassermann test at all, unless a sensitive method is employed, because the results are almost sure to be misleading in the way of giving a false sense of security. *Even when the blood Wassermann tests with a sensitive method yield a series of negative reactions, we should advise at least one examination of the spinal fluid for total cells, protein, complement fixation and colloidal gold reactions before dismissing the case as cured in order to avoid the regrettable error of stopping treatment in the presence of residual infection of the central nervous system.* Such experience syphilologists as Fordyce, Rosen, Moore, Keidel, Stokes, Gennerich, etc., have laid particular emphasis upon the thorough treatment of early syphilis is the best means for preventing neurosyphilis. Gennerich[29] has even advised combined intravenous and intraspinal treatment in secondary syphilis; Picard[30] and others have drawn particular attention to the danger of insufficient treatment in relation to infection of the central nervous syphilis and the necessity for intensive specific medication, not with pills and powders, but with our best spirocheticides. In Germany and Austria some syphilologists are even employing malarial inoculations in secondary syphilis for the removal of syphilitic infection of the central nervous system.

Doubtless some cases of early syphilis respond more promptly than others; so many factors influence the disease, as virulence of the infection, hereditary disposition to neural infection, occupational and other injuries favoring localization of the spirochetes, etc., that no one program of treatment can be applied to all cases but it would appear advisable for every physician treating early syphilis to insist upon a certain minimum amount of treatment regardless of how quickly clinical and serological evidences of cure are obtained. For my own part, I believe that the treatment outlined in paragraphs 1 to 12 under the method of treatment for primary syphilis, constitutes the minimum for cases of primary and secondary syphilis with positive complement fixation reactions. I realize that this amount of treatment may be more than required for some cases and especially primary cases in the first ten days with negative Wassermann reactions, but I prefer to possibly overtreat some cases to undertreating a single one if this regrettable error is at all avoidable. Objection may be raised on the matter of expense but few physicians will permit this to interfere with their advice and services. There should not be a fixed scale of prices but the charge arranged according to the financial status of the patient to make possible the prolonged and thorough treatment required. The outrageously high fees demanded some years ago for a "shot" of arsphenamin sometimes making it impossible for the victim to afford a second or third, have no place in the present day treatment of the disease when not one or two, but twelve or more injections, are required as a minimum.

Criteria of Cure of Early Syphilis.—Finally the most important question of all is to decide when treatment may cease; I say *treatment* because I believe *observation* should continue much longer. We should never fail to explain the disease to the patient and the utter impossibility of our being able to state in definite and absolutely safe terms that a radical cure has been accomplished in the sense of complete eradication of every syphilitic spirochete from the body. The patient should know this and if any symptoms or lesions develop in the future not otherwise to be accounted for, he should acquaint his physician with the fact of having contracted syphilis. There are, however, different ways of discussing the subject with the patient: one is the gloomy, pessimistic way tending to create those regrettable incurable "syphilitic scars of the spirit," and it should never be employed. Another is the careless, off-hand, cock-sure way of stating that a cure has been accomplished as certain as death and taxes; it is apt to render some persons careless of their future welfare and it is very frequent indeed, to see individuals with advanced chronic lesions of syphilis who neglected seeking advice for long periods of time and who are dumb founded when informed of the nature of their complaints, because of having been previously assured by their physician of complete cure in some such dogmatic manner.

I believe available evidence indicates that syphilis adequately treated in the primary and secondary stages is frequently cured in the complete and radical meaning of the term and not merely "whitewashed" over; but it will require another ten to twenty years before the results of present day methods can be evaluated in final terms. But I believe our modern methods are at least superior to the older methods of treatment; cures were apparently accomplished by treatment with mercury alone but not nearly as frequently as Ricord, Fournier, Hutchinson and other experts long since gone to their reward for good work well done believed, when modern methods of diagnosis are applied to individuals treated according to their methods and especially examinations of the cerebrospinal fluid and central nervous system.

This subject, however, is discussed at more length in Chapter LXXIX; here we are concerned only with what may be called the criteria of cure after the treatment of acute early syphilis. Only an opinion as follows can be expressed subject to future modification:

1. At least one year of treatment with arsphenamin or its substitutes with mercury or bismuth; at least six years of treatment with mercury and iodids if these alone are employed.

2. The total disappearance of all clinical evidences of syphilis as determine by a thorough physical examination including an examination of the central nervous system.

3. At least one year and preferably two years of observation following the cessation of specific treatment or with occasional short courses of treatment during which four or more blood Wassermann tests with a *sensitive* method have yielded consecutively negative reactions.

4. At least one examination of the spinal fluid during this probationery period in which the pressure is not increased, the total cells not more than 10 per c. mm., the protein tests negative, the Wassermann tests negative with large amounts of fluid and the colloidal gold test negative.

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CHAPTER LXXIII

SPECIFIC TREATMENT OF LATENT, WASSERMANN-FAST AND CHRONIC ASYMPTOMATIC SYPHILIS

The most peculiar and least understood of all phases of syphilis are the periods of remission or latency. In cases of untreated syphilis a period of latency follows the secondary stage of one to ten years or longer, usually two to five years, during which the patient may feel and appear normal and healthy; this is called the *early latent period*. In cases of insufficiently treated early syphilis, this latent period is likely to be of longer duration and give a false sense of security to a large proportion of syphilitics. Even after the close of this period when tertiary lesions have developed, periods of remission or latency may occur in the tertiary stage in which lesions subside and the disease is quiescent; these are designated as *late latent periods*. The purpose of this chapter is to discuss specific therapy in cases of early and late latency, as it concerns a very large proportion of syphilitics and offers peculiar and difficult therapeutic problems.

Nature of Latency in Syphilis in Relation to Specific Therapy.—I have already discussed the general nature of latency in Chapter XXXVII; what little we know of the mechanism indicates that the parasites are thrown into a state of reduced activity either by immunological processes, by changes in the life cycle of the organisms, or by a combination of the two. Available evidence indicates that an immunological mechanism is chiefly concerned which is peculiar in that it is largely due to resistance offered by the cells in direct contact with the spirochetes rather than to antibodies in the blood. This cellular resistance is not in the nature of phagocytosis; indeed, there is very little evidence to indicate that phagocytosis plays any important part in the immunity of syphilis. Rather the cells develop some kind of intracellular

change or resistance analogous to the intracellular changes occurring in anaphylaxis. These changes appear to occur only in the tissues directly infected by *Sp. pallida,* rather than in distant organs like the bone marrow, so well known to be an active producer of antibodies in most of the bacterial infections.

Clinical versus Pathological and Serological Latency.—Are these immunological changes curative in the sense of destroying spirochetes? Very probably large numbers of the organisms are actually destroyed, but I believe that complete radical cures are of rare occurrence. It cannot be denied, however, that many syphilitics and especially those in latency following six months or more of treatment in the early stages, live to a ripe old age in fairly good health. This is especially true of syphilitic women who have borne children and is more likely to occur among the colored of both sexes than among the whites. Even in the skin and mucous membranes where the curative activity of these immunological processes is most marked, as shown by the spontaneous healing of the chancre and secondaries, complete eradication of spirochetes may not occur as shown by the occurrence of recurring chancres (chancre redux) in the scars of former chancres and the development of condylomata and even recurrent acute secondaries in about 20 per cent. of untreated cases of early syphilis. It may be that the recurring acute skin lesions are due to periods of spirochetemia, but condylomata and chancre redux are almost certainly due to the renewed activities of spirochetes persisting in the tissues from the time of their initial lodgement.

But aside from these evidences of continued spirochetic activity, there may be no other clinical manifestations during the early latent period of syphilis. The patient may and usually does feel a sense of well being which leads not a few into a false sense of security with neglect of further observation and treatment. But are the spirochetes escaping destruction truly latent? Unquestionably their rate of multiplication and virulence are held in check during this period, but the survivors wage unceasing combat with the immunological forces of the tissues and, in the majority of cases of untreated or insufficiently treated syphilis, slowly but surely make progress during periods in which the equilibrium between infection and resistance is unbalanced in favor of infection, and are especially likely ultimately to produce lesions in the cardiovascular and central nervous systems. But during the first two to ten or twenty years, the lesions may be so small or so situated that symptoms and signs of disease are not apparent, although the incidence or percentage of detection of these depends upon the thoroughness of examination and the skill of the physician. But nevertheless the lesions may be present and this constitutes the great difference between what may be called "clinical latency" and "pathological latency." The former is frequent because of fortuitous circumstances preventing the detection of lesions which may be of microscopic size; the latter is much less frequent in that the spirochetes, while held in check, are not in complete check or latency but slowly and surely making progress as shown ultimately by the production of the multitude of lesions embracing the tertiary stage of syphilis.

Furthermore during these periods of clinical latency the Wassermann reaction may be positive and, indeed, constitute the only sign of persistent syphilitic infection. In the presence of apparent good health, and especially over a period of years, the perfectly natural tendency is to regard positive reactions as due to errors in technic or some cause other than the only true one—syphilis. This is one of the most serious mistakes being made to-day by both physicians and the laity. I do not know of a single reason for the occurrence of technically correct and truly positive Wassermann reactions

under such conditions except syphilis, when the matter of frambesia, and, possibly, leprosy, can be excluded. I have, however, already discussed this subject at length in Chapter XXXIX, but after many years of the closest study of the complement-fixation test in relation to syphilis, I wish to warn with all the strength at my command, against the custom of ignoring positive reactions in cases of clinically *concealed* syphilis. I freely admit that these reactions may be sometimes due to errors in technic, but the tests should be at least repeated before being ignored. Even weakly positive reactions are not to be ignored and any serologist who doubts the accuracy of weakly positive reactions should abandon his method for a better one. Even under the best of circumstances the most sensitive methods may still err on the side of yielding falsely negative reactions, because the syphilitic lesions are so small that not enough "reagin" is being thrown into the blood to enable detection in the test-tube. There is, therefore, a great difference between "clinical latency" and "serological latency." By means of a reliable complement-fixation test possessing the maximum degree of sensitiveness compatible with practical specificity, we are able to detect evidences of continued or renewed spirochetic activity in a larger, a very much larger, percentage of cases than is possible clinically. In other words, the positive Wassermann reaction by an acceptable method constitutes one of the most sensitive signs of concealed or latent syphilis frequently apparent months and years before lesions have grown to such size or developed in such organs as to produce such signs and symptoms as may produce ill health and lead to their clinical detection. I repeat again and again, that *to ignore this sensitive sign of syphilis is a serious mistake;* it should at least lead to further serological tests and more thorough and exhaustive examinations of the patient.

Interruption of Latency by Trauma, Age and Treatment in Relation to Specific Therapy.—If an indefinite period of clinical latency could always be counted upon in syphilis, the question of treatment would be much less important but there is always the danger of the disease springing into renewed activity. We do not know in exact terms what may be the determining factors, but evidence indicates that anything lowering the vitality or resistance of the host, either at a local point or in general, favors a cessation of latency and renewed spirochetic activity. Tissues or organs the seat of over-work and strain or physical trauma are especially apt to develop active lesions. Furthermore, the wear and tear of life, its activities and age tend to break the equilibrium between syphilitic infection and resistance in favor of the spirochetes. It may be that an individual will reach a ripe age before such occurs, but the break is so likely to occur that syphilis in the latent stages is worthy of therapeutic attack if for no other reason, that to lengthen the period of latency and thereby prevent the development of crippling lesions as long as possible, even though complete and radical cure may not be attainable. This, however, like everything else, should be subject to exceptions and the exercise of common sense. For example, in those who have reached the age of 60 years or thereabouts and who are in good health, it may be a matter of wisdom to leave well enough alone; their organs, and especially the kidneys, liver and skin, may not be able to eliminate drugs sufficiently well and thereby render our therapeutic efforts more dangerous than the disease and therefore of doubtful value. Furthermore, their vital organs may be crippled by other infections and render them less able to combat the toxic or organotropic effects of our spirocheticidal drugs. But most important of all, our antisyphilitic drugs may actually stimulate the spirochetes into such activity that their progress is hastened rather than checked (Herxheimer reaction). But among the younger and especially that big class of both sexes

between 35 and 55 years of age, who contracted syphilis ten or more years previously with or without treatment at the time, the question of judicious specific treatment during clinical latency is a matter of importance and in my opinion, is always advisable as "a stitch in time to save nine." And frequently the very first sign of this latency during which so much may be done for the unfortunate victim in the way of *judicious* advice as to the manner of living and specific therapy, is the much maligned, frequently misunderstood and underrated complement-fixation text by an acceptable method!

Principles of Specific Therapy of Latent Syphilis.—How may the dangers of therapeutic Herxheimer reactions be avoided? Largely by starting treatment with the mercurials, bismuth or intramuscular injections of sulpharsphenamin. But may not these compounds have a similar action to the stimulating effects of intravenous injections of arsphenamin and its substitutes? Yes, they may and especially if administered in large doses by intravenous or intramuscular injection but not nearly as much as intravenous injections of the organic arsenicals. But cannot the same effects be secured by instituting treatment with small (0.05 to 0.1 gm.) doses of arsphenamin or neoarsphenamin? Yes, but less efficiently than with mercury and bismuth largely because of the more rapid elimination of the arsenicals. Mercury is less rapidly converted into inert compounds and less rapidly eliminated; I believe it tends to penetrate chronic lesions and localize in them to a better degree than the organic arsenicals, gradually and finally reaching a degree of spirocheticidal concentration even though the blood in general may at no time contain a spirocheticidal concentration. This, I believe, is the secret of success of mercury and bismuth in the treatment of chronic syphilis either active or in the latent phases. The organic arsenicals also tend gradually to penetrate chronic lesions and especially if the rate of oxidation and elimination are reduced by intramuscular injections; but clinical experience shows that Herxheimer reactions can be prevented in most cases by instituting treatment with small doses of mercury or bismuth and the iodids, or small doses of the organic arsenicals before large doses of any are given. Why this is so cannot be stated in definite terms, but it is my opinion that mercury, bismuth and arsphenamin or its substitutes have a special selective affinity for syphilitic exudates and when given in small doses tend to accumulate in the lesions where they finally reach such a concentration as effectually prevents or controls renewed spirochetic activity and eventually brings about spirochetic destruction.

Clinical experience indicates, therefore, that the treatment of latent syphilis should be begun with mild rather than intensive methods, in order that the advance scouts of a therapeutic attack may be sneaked into the strongholds of syphilitic infection to overcome the spirochetes and hold them in check when the heavy attack is opened up. The iodids are good auxiliary scouts for opening up the gates of the forts for the attacking forces in the way of softening or breaking down the organized exudates of the foci. This is true of the treatment of both early and late latent syphilis but especially of the latter. In early latent syphilis and especially when treatment is re-instituted during the first two years after infection following an earlier course of treatment, as when the Wassermann test becomes positive in the second probationery year after having been previously negative, therapeutic Herxheimer reactions are less likely to develop because it is probable that sufficient arsenic and, particularly, mercury have been retained to restrain sudden renewed activities of the spirochetes. But in late latent syphilis this is not likely and, furthermore, the spirochetes may have had sufficient time to acquire enough fastness or resistance to arsenic to counteract the spirocheti-

cidal activity of arsphenamin and its substitutes even though they may be sufficiently sensitive as to be stimulated by them.

After treatment has been begun in this mild manner, later treatment may be as vigorous as advised for the intensive treatment of early syphilis. This, however, is subject to wide variation according to individual cases. In latent syphilis of the young or middle aged possessing a good pair of kidneys and other vital organs in good shape we may follow the opening attack with fairly active therapeutic measures, but whenever there are reasons for surmising involvement or dysfunction of these, as is frequently the case in the aged, it is better to continue with milder treatment over longer periods of time.

Nature of Wassermann-fast Syphilis in Relation to Specific Therapy.—Before, however, taking up the actual treatment of latent syphilis, it may be well to briefly discuss the subject of Wassermann-fastness in relation to specific therapy and especially since this condition is frequently met with among individuals in late latent syphilis showing no detectable lesions or symptoms of the disease. There is probably no subject more perplexing than the clinical and pathological significance of this condition and especially from the standpoint of specific treatment. Wassermann-fastness refers to individuals who have received thorough courses of specific treatment, who feel well and in good health, who may show no clinical signs or symptoms of syphilis but whose blood Wassermann tests yield persistently positive reactions when conducted by acceptable methods. Are these individuals really syphilitic? Should treatment be instituted or may the positive Wassermann reactions be safely ignored? There is a wide divergence of opinion upon these questions and another ten or twenty years will be required to give the answers according to the final fate of those to whom treatment is given and not given. But what is the evidence available at present, as these questions involve the happiness and lives of a very large number of individuals?

In the first place, when are we justified in regarding a patient as Wassermann-fast? The criteria vary greatly in the practice and opinions of different syphilologists. What one physician may regard as a case of Wassermann-fastness because the reaction is positive after a certain amount of specific treatment—say, twelve injections of arsphenamin along with courses of mercury—may not be so regarded by another. Furthermore, one physician may meet with the condition but rarely and ascribe this to some superior quality of his methods for treating syphilis, whereas, in truth, he is being deluded by Wassermann tests lacking in sensitiveness. Again a physician may believe he is dealing with Wassermann-fastness because the tests are conducted with a single amount of serum, as 0.1 or 0.2 c.c. which do not show a possible quantitative decrease of "reagin" or Wassermann antibody nearly as well as quantitative Wassermann tests employing varying amounts of serum, as in my new quantitative method. It is impossible, therefore, to define what constitutes Wassermann-fastness unless we adopt arbitrary standards and my own criteria are, cases whose blood Wassermann tests by my method yield persistently positive quantitative reactions after prolonged and thorough treatment: in early syphilis as described in the preceding chapter; in early latent syphilis as described in this chapter. It is impossible to express the incidence of Wassermann-fast syphilis, because of these wide variations in treatment and sensitiveness of serological tests, but it is safe to say that the condition is not uncommon and sometimes constitutes one of the most perplexing problems in the management of syphilis.

But this is only so when thorough physical and roentgenological examinations fail to disclose evidences of syphilis. As discussed at greater length

in succeeding chapters, thorough examinations of the cardiovascular, central nervous, gastro-intestinal systems, etc., usually disclose clinical evidences of syphilis and it is safe to state that thorough microscopical examinations of the tissues after death of cases of this kind in whom no clinical evidences of syphilis were detectable during life, will disclose evidences of the disease in one or more organs. In the study made by Stokes and Busman of 458 Wassermann-fast syphilitic patients, there were only 10 patients or about 2 per cent. presenting no other evidence of syphilis than their positive Wassermann reactions. The question is really not one of whether these cases are syphilitic, but rather how should they be managed therapeutically? Indeed, Wassermann-fastness is not only a sign of syphilis but usually a sign of progressive chronic syphilis rather than of truly latent syphilis. Therefore, these cases generally require something in the way of specific treatment and it is my practice to advise prolonged specific medication after the plan described in this chapter; I have not yet encountered a case which has not shown some response, as detected not only clinically but more especially serologically with my quantitative complement-fixation test. I really doubt if any but exceptional cases of syphilis are absolutely Wassermann-fast, although in my experience some cases and, especially cardiovascular cases of acquired syphilis and late congenital infections among adolescents and adults, have required as much as five years of persistent effort gradually to remove this state. But, is it advisable and necessary to give treatment merely to remove Wassermann-fastness? Since I believe that Wassermann-fastness is a sign of syphilitic infection and usually progressive rather than truly latent infection, I believe it is whenever our patient is less than 60 years of age and in good general condition permitting the administration of antisyphilitic drugs over a long period of time without injury to the tissues. In elderly individuals however, I believe it is sometimes sufficient to leave well enough alone while maintaining a policy of watchful waiting.

Methods of Specific Treatment of Latent Syphilis.—While many writers have proposed various plans or methods for the treatment of early syphilis, as stated in the preceeding chapter, the literature contains relatively few contributions to the present subject except for some articles discussing the subject in a broad and general manner with the general consensus of opinion that thorough serological tests of blood and spinal fluid and clinical examinations should be made with the institution of specific medication as insurance against dangerous or incurable conditions in later life. One is impressed, however, with the haziness of opinions and the therapeutic measures to be adopted. It is not to be expected, however, that definite plans can be proposed, except possibly for early latent syphilis when we may still treat the disease rather than the patient in many instances. In late latent syphilis so many complicating factors require consideration that only the general principles and examples may be given as in the treatment of chronic active syphilis. The majority of writers appear to be in favor of mixed treatment with arsphenamin and its substitutes, mercury and the iodids. Corbus, for example, in the treatment of latent cases with positive blood Wassermann reactions and negative spinal fluids, advises 50 grains of potassium iodid daily for four weeks, followed by eight weekly injections of arsphenamin, potassium iodid again for four weeks, eight more weekly injections of arsphenamin and eight weeks of daily inunctions of mercury.

Author's Method of Specific Treatment of Early Latent Syphilis.—From the standpoint of specific therapy, early latent syphilis may be divided into two classes as follows: (1) Cases of *untreated* early syphilis in the period of latency following the primary and secondary stages and (2) cases of *treated*

early syphilis in whom positive Wassermann reactions occur during the two or three years following the chancre.

In the *first group* are those individuals who contracted syphilis without suspecting the disease. The chancre may have been so small that it was regarded as a simple non-syphilitic lesion or it may have been hidden from the view or so situated as on the lip or finger, as failed to excite suspicion of its true character. The secondaries may have been light and fleeting. Women are especially likely to fall into this class; also physicians unsuspecting the true nature of an extragenital infection. These cases are not at all infrequent and are usually discovered more or less accidentally by the Wassermann test or clinically by the history, the presence of venereal warts, sore throat, etc. As a general rule the Wassermann reaction is strongly positive and there are reasons for always suspecting a heavy infection and particularly of the central nervous system, cardiovascular system or other organs, although most searching physical examinations may fail to disclose lesions because these cases occur within a two-year period of the time of infection according to this classification. It is not possible to lay down sharp, definite rules of treatment applicable to all cases, because of variations demanded by individual cases, but thorough treatment is indicated somewhat after the following plan (the treatment of exceptional and difficult cases being considered in Chapter LXXVIII):

1. Careful instruction regarding the infectiousness of any open lesions that may occur and especially in the mouth; also in the case of males, the infectiousness of the semen and the strong possibility and probability of transmission to both woman and child in pregnancy.

2. Instruction in general hygienic care, as described in Chapter LXIX.

3. Examination of the urine and the institution of specific medication.

4. If there are no open lesions it is generally advisable to initiate treatment with daily inunctions of one dram of 50 per cent. mercurial ointment or 85 per cent. calomel ointment along with 20 to 40 grains of potassium iodid daily by ingestion for two weeks; this reduces the chances of producing neural Herxheimer reactions.

5. If open lesions are present, as venereal warts or mucous patches, it is well to start at once with the organic arsenicals beginning with small doses and increasing at five to seven day intervals. It is also permissible to initiate treatment with the organic arsenicals in cases without open lesions, but always with small doses at first to reduce the danger of occurrence of Herxheimer reactions.

6. Neoarsphenamin may be given by intravenous injection, beginning with 0.4 gm. and increasing the dose if all goes well until 0.9 gm. is being administered by the fourth injection; at least twelve injections should be given for the first course. If arsphenamin is administered, at least six injections may be given starting with 0.3 gm. If sulpharsphenamin is given, at least eight epifascial injections of 0.4 gm. each may be given.

7. Immediately after the last injection of arsenical, a compound of mercury or bismuth and the iodids should be given for a period of two months. Daily inunctions of mercurial or calomel ointments are preferred along with the oral administration of potassium iodid. If inunctions cannot be prescribed potassium tartrobismuthate may be given by intramuscular injection every five days in dose of 0.2 gm. each, totalling about twelve injections or 2.4 gm. in the two months. Or mercury succinimid or the salicylate may be given in dose of 1 to 1½ grains once a week by epifascial injection, totalling eight injections for the two months. The urine should be examined every two weeks.

8. A rest of one to two months without specific medication may now follow; if, however, the kidneys and general condition are satisfactory, it is well to continue mercurial treatment during this period in the form of 1 grain of protiodid daily in divided doses. A Wassermann test may be made at the end but regardless of whether the reaction is positive or negative treatment is continued.

9. The series of injections of arsenical is now repeated, the balance of treatment for the one to two years being after the method advised for the treatment of secondary syphilis described on page 957 including blood Wassermann tests, cerebrospinal fluid examinations and the same criteria of cure described on page 960. As a general rule more treatment is required for syphilis in this stage than in the secondary stage, because of the longer duration of infection before the institution of treatment.

Cases of the *second group* may occur when the treatment given in the primary and secondary stages of syphilis has been insufficient. As a general rule, there are no discernible open lesions, indeed nothing to indicate persistent syphilitic infection except positive blood Wassermann reactions following one or more negative reactions. Not infrequently, however, spinal fluid changes are likewise present in the form of a weakly positive Wassermann reaction, a colloidal gold reaction of 0 1 2 2 1 0 0 0 0, 2 2 1 0 0 0 0 0 0 or some modifications of these, as well as a slight increase of cells and protein. Unquestionably treatment should be resumed because these are indications of syphilitic infection, possibly of latent infection, but more probably of mild, progressive infection of the cardiovascular, neural system or other organs by spirochetes escaping destruction during the first six to twelve months of treatment. As a general rule the majority of cases of this group occur in the second or third years after infection following a year of treatment and are detected by the routine Wassermann tests advised during this probationery period. Should the treatment be mild as the administration of mercury and iodids by mouth or more vigorous? In my opinion spirochetic activity of sufficient degree to produce a positive blood Wassermann reaction is deserving of fairly active treatment unless contraindicated by complicating factors in certain individuals. As a general rule, this consists of alternate courses of arsenicals, mercury or bismuth, with the iodids, somewhat as follows:

1. The first course if of mercury by daily inunction for one month or an intramuscular injection of potassium tartrobismuthate in dose of 0.2 gm. every five days for eight injections or an epifascial injection of mercury succinimid or salicylate in dose of 1 to 1½ grains every week for four injections. At the same time potassium iodid is prescribed by ingestion in amount of 20 to 60 grains daily in divided doses. This mild initiating course is especially advisable when more than four months have elapsed since the last previous course in order to reduce the chances of producing Herxheimer reactions.

2. This course may be followed by a series of twelve weekly intravenous injections of neoarsphenamin or six of arsphenamin; or eight epifascial injections of sulpharsphenamin at weekly intervals.

3. Beginning immediately after the last injection, daily inunctions of one dram of mercurial ointment or 85 per cent. calomel ointment are prescribed for a period of two months; potassium iodid is given at the same time in a well-tolerated dose. If for any reason the inunctions cannot be given, I give alternate intramuscular injections of potassium tartrobismuthate in oil in dose of 0.2 gm. and succinimid of mercury in oil in dose of 1 grain, once a week for three months (6 injections of each). If mercury cannot be given, I give twelve doses of the bismuth preparation (a total of 2.4 gm.).

4. This course is followed by one month of rest.

5. Mercury is then given by mouth for three months, usually in the form of ⅓ grain of the protiodid or 1 grain of mercury with chalk three times a day after meals.

6. The patient is instructed to bring urine for examination every two or three weeks throughout which also permits one to examine the mouth, etc., for evidences of mercurialism, iodidism, etc.

7. The Wassermann test is now conducted. If positive, these courses or some slight modification of them are repeated. If negative, another short course of arsenic is given (six weekly injections of neoarsphenamin or four of arsphenamin) followed by three months of mercury and bismuth or bismuth alone, etc., as described above in paragraph 3 of this section. This is followed by one month of rest, three months of mercury by mouth and a Wassermann test.

8. In cases of this kind the prognosis must be guarded and the criteria of cure more rigid than usual. I never sanction marriage until the blood Wassermann tests have been negative consecutively over a period of at least one and preferably two years, during which at least one examination of the cerebrospinal fluid has yielded negative results.

9. After the second negative Wassermann reaction has occurred (I do not like the word "secured" because it gives a false impression that this is the sole aim of treatment which is far from being true), mild treatment is advisable during the following one to two years, somewhat after the plan of Fournier's chronic intermittent treatment, namely, alternate courses of two months of mercury and iodids by mouth followed by two months of rest (page 1050).

Author's Method of Specific Treatment of Late Latent, Chronic Asymptomatic and Wassermann-fast Syphilis.—Late latent syphilis is applied to those periods of *clinical* latency developing two to ten, or more years after infection. The patient may appear and feel well although in the majority of cases a thorough physical and roentgenological study discloses evidences of chronic syphilis in one or more organs. These lesions, however, are apparently quiescent although the serological tests of blood and spinal fluid are likely to yield positive reactions. This constitutes clinical latency, because, pathologically, the disease may not be truly latent but insidiously progressive; neither is it serological latency, if quantitative complement-fixation tests show progressively stronger positive reactions in both blood and spinal fluid. In many cases, probably the majority, the diagnosis is made only by the Wassermann test frequently first applied in a more or less accidental manner in the course of routine examinations for life insurance or the diagnosis of some chronic ailment as vague neuritic pains, disturbances of vision or hearing, chronic nephritis, diabetes, cardiac pain, and a host of other ailments bringing the middle aged to the physician for advice. Women who have borne syphilitic children frequently belong to this group, the diagnosis being first made in the course of Wassermann and clinical surveys of a family when evidences of late congenital syphilis are discovered in one or more children. Congenital syphilis of adolescents and adults are likewise generally included in this group, because, while clinical evidences of syphilis may be found, the lesions are apparently quiescent. It includes, therefore, a very large and very important group of syphilitic individuals including those who may be designated as Wassermann-fast cases.

Should specific treatment be instituted in cases of this kind when the only evidences of syphilis may be a history of doubtful significance or value with positive blood Wassermann reactions? I believe that treatment is indicated in the majority of cases in order to leave nothing undone that may be safely

done to prevent the development of chronic lesions in later years that may attack any organ or part of the body and especially the most vital organs of all, as the central nervous system, cardiovascular system, liver, etc.

The first thing to be done is to repeat the Wassermann tests, examine the spinal fluid in some cases and subject the patient to a rigid physical examination especially of the central nervous and cardiovascular systems. If the serological tests of the blood or spinal fluid or both yield positive reactions, I believe that treatment should be instituted even though the results of clinical examinations are negative or doubtful, if the patient is less than 60 years of age and in such condition to render fairly safe the administration of antiluetic drugs in adequate doses. No one plan of treatment, however, is applicable to all; pathological changes in various organs as nephritis, tuberculosis, etc., may require important modifications as discussed in Chapter LXXVIII. Furthermore, cases of this kind frequently display intolerance of various degrees for the organic arsenicals rendering necessary an arrangement of doses and intervals for each case and not infrequently an entire rearrangement of a therapeutic program.

If in addition there are clinical evidences of chronic syphilis of one or more organs, the indications for specific treatment are just so much stronger. But, as just stated, the method of treatment is subject to many variations, according to individual cases; we cannot treat the disease alone, but rather the patient. For my own part, the treatment is essentially that of chronic syphilis with signs and symptoms of the disease. In other words, I prefer to advise and manage cases of *late clinically latent syphilis* with persistently positive Wassermann reactions in exactly the same manner as cases of chronic syphilis showing evidences of progressive lesions. It is true that some may not require as much treatment as the latter and may give a better prognosis, but the underlying principles and treatment are essentially as described in the succeeding chapter.

The plan of treatment herewith submitted is used primarily for individuals under 60 years of age as a means for gradually eradicating the infection or so reducing it as to practically insure a prolonged period of latency amounting almost to the same thing. For older individuals a similar plan may be employed, but generally modified to render it milder and safer from the standpoint of all effects. This plan is as follows:

1. A careful and complete physical examination is made, including an examination of the urine. As a general rule, however, no weak links are found in this class, but in Wassermann-fast cases it is especially important to determine carefully the condition of the liver and kidneys in view of the possible effects of previous treatment.

2. A careful search is made for focal infections with extirpation or correction of these. The patient is instructed in the care of the mouth and general hygiene; the use of alcohol is forbidden and smoking greatly reduced if necessary. Every effort is made to improve or maintain good general health, as free as possible of sustained physical or mental strain.

3. The first course of specific treatment is usually of mercury by inunction and potassium iodid by ingestion for two months. These are to be preferred to intravenous injections of arsphenamin or its substitutes in order to minimize the risks of producing Herxheimer reactions.

Each application of the mercury is one dram of the official 50-per cent. ointment or 85-per cent. calomel ointment; the iodid is begun in daily dose of 15 grains and gradually raised, if possible, to at least 45 grains per day. If inunctions cannot be given, I administer intramuscularly 0.2 gm. or potassium tartrobismuthate in oil or 1 grain of the salicylate or succinimid of

mercury in oil once a week for the eight weeks. If for any reason the injections cannot be given, mercury may be administered by ingestion in dose of ¼ grain of the protiodid four times a day. The urine is examined routinely at frequent intervals and a careful watch maintained for toxic effects from mercury or bismuth and the iodids.

4. This course is followed immediately by one of arsenic. Neoarsphenamin may be given by intravenous injection once a week for twelve weeks, beginning with 0.45 gm. and gradually increasing until 0.9 gm. is given in the fourth and subsequent injections. Sulpharsphenamin is second choice in dose of 0.4 to 0.6 gm. once a week for ten weeks. If arsphenamin is administered the first dose is 0.2 gm. with gradual increases until 0.4 to 0.6 gm. are being given; the course consists of at least 8 injections. My present plan is to alternate intravenous injections of neoarsphenamin with epifascial injections of sulpharsphenamin, giving 8 injections of each over a period of sixteen weeks. This permits the intramuscular reactions to subside and the results, judged largely by the Wassermann tests, have been very gratifying and encouraging. Conrad and McCann[1] write of excellent results following 3 intravenous injections of arsphenamin at weekly intervals along with 20 intramuscular injections of mercuric chlorid at 2–3 day intervals beginning with 10 minims of a 1-per cent. solution in saline; this dose is increased by 2 minims at each treatment until 2 c.c. is given at one time. Advantages claimed for this method of administering mercury are the more accurate grading of doses and practically painless injections.

5. If the treatment is being well borne, it is continued in the form of two more months of daily inunctions of mercurial or 85-per cent. calomel ointment in amount of one dram, along with potassium iodid by mouth.

Up to this point the treatment was begun mildly to prevent Herxheimer reactions and followed up by rather intensive treatment to overcome or check renewed spirochetic activity. From now on the balance of specific treatment may be of milder degree.

6. A rest of two months without specific medication is given or the patient may receive ½ to 1 grain of protiodid of mercury daily in divided doses along with tonic and general medication if required or indicated. A quantitative Wassermann test is then conducted.

7. From now on I am inclined to administer a mixed or combination type of therapy. For two months potassium iodid is given by mouth with three inunctions of mercurial or calomel ointment per week. Each week neoarsphenamin (0.6 gm.) is injected intavenously or, if the patient permits, alternate intravenous injections of neoarsphenamin and epifascial injections of sulpharsphenamin are given. If sulpharsphenamin is refused, 0.2 gm. of potassium tartrobismuthate is given once a week by intramuscular injection. Of course, the urine is carefully examined every two weeks, but I have rarely found it necessary to alter this plan on account of renal irritation.

8. This completes the first year of treatment. If the clinical condition of the patient is good and the blood Wassermann reactions are negative, an examination of the spinal fluid is made. If the results are negative, the patient is put upon ⅓ grain of protiodid of mercury or 1 grain of mercury with chalk with 1 grain of Dover's powder three times a day for three months. This is followed by two months of rest, three months of mercury, two months of rest, etc., for the next three years, very much after the plan of Fournier's chronic intermittent treatment described on page 1050.

9. A clinical examination and a blood Wassermann test are made after each rest period, the patient, however, reporting at frequent intervals during the periods of treatment to be looked over for signs of mercurialism and for

urine examinations. If the Wassermann reaction has remained positive or becomes positive, more energetic treatment is given, consisting of a repetition of the course described in paragraph 7 followed by two months of mercury by mouth, two months of rest, two more months of injections, etc., finally winding up as described in paragraph 8.

10. In Wassermann-fast cases I sometimes alter the plan given in paragraph 7 by injecting intravenously with a syringe a mixture of 0.6 gm. of neoarsphenamin in 10 c.c. of water plus 10 c.c. of a 2-per cent. solution of flumerin (0.2 gm.) or 10 c.c. of 1: 1000 solution of mercurophen (0.01 gm. or $\frac{1}{6}$th grain) once a week alternatingly with intramuscular injections of 0.2 gm. of potassium tartrobismuthate in oil. At the same time potassium or sodium iodid are given in maximum tolerated doses by ingestion.

REFERENCE

1. Arch. Dermat. and Syph., 1922, 6, 50.

CHAPTER LXXIV

PRINCIPLES OF THE TREATMENT OF CHRONIC SYPHILIS

Dissemination of Lesions in Chronic Syphilis in Relation to Treatment. Probably the best known form of chronic syphilis is of the central nervous system by reason of the large amount of attention devoted to this subject within the last ten years and especially from the standpoint of various forms of intraspinal therapy. But there is a growing appreciation of the fact that a large percentage of neurosyphilitics also show chronic syphilitic lesions of the cardiovascular system as well and sometimes of the liver and other organs. It is the possibility and probability that important lesions may be present in several organs of vital importance that requires a consideration of the patient as a whole rather than concentration alone on the organ presenting the most active lesions and symptoms, when the treatment of chronic syphilis is undertaken. Why and how *Sp. pallida* selects certain organs and tissues when present in the blood and lymph and carried equally to all parts of the body, cannot be definitely stated; some believe it is a matter of selective strains of the parasite having an elective affinity for certain organs and tissues, but I have discussed this subject with detail in Chapter XXXVI and expressed the opinion that it was not so much a matter of specific strains but rather one of hereditary or acquired injury or predisposition of certain organs and tissues in the nature of areas of reduced local resistance which constituted the chief factors influencing the localization of *Sp. pallida.* Furthermore, I believe it is a mistake to subscribe to the view that the only time of dissemination of *Sp. pallida* is in the early stage of syphilis and that the ultimate fate of the syphilitic is determined at this time according to where the parasites find lodgement during the spirochetemia occurring between the chancre and the outbreak of the secondaries; doubtless this is an important time, but I believe that temporary periods of spirochetemia may occur in the later stages of the disease as well during which the parasites may find lodgement in the tissues injured by over-work, strain and the wear and tear of life subsequent to initial infection and the primary spirochetemia. It is for this reason that advice on the proper manner of living and working

is of importance to every case of early syphilis, in order to reduce the chances of trauma in a broad sense as much as possible.

One of the most instructive chemical studies on the dissemination of lesions in chronic syphilis, is that of Stokes and Busman,[1] of 458 syphilitic patients who had received from twelve to twenty-nine arsphenamin injections combined with mercurial inunctions and all of whom yielded positive Wassermann reactions. In this survey the authors had the great advantage of cooperation of specialists in the neurologic, eye, nose and throat, roentgenologic, laboratory, and other divisions of the Mayo Clinic and the results serve as an excellent example of the practical value of coöperation in the diagnosis of chronic syphilis; the occurrence of multiple lesions in 114 of these patients was as follows:

Of 15 patients with cardio-vascular syphilis:
20 per cent. also had neurosyphilis.
20 per cent. also had osseous syphilis.
20 per cent. also had cutaneous and mucous membrane syphilis.
6 per cent. also had heredosyphilis.
6 per cent. also had splenic, hepatic or gastric syphilis.

Of 21 patients with neurosyphilis:
50 per cent. also had cardio-vascular syphilis (of 6 specially examined).
18 per cent. also had osseous syphilis.
14 per cent. also had splenic, hepatic or gastric syphilis.
4.6 per cent. also had cutaneous syphilis.

Of 30 patients with osseous syphilis:
43 per cent. also had cardio-vascular syphilis (of 6 specially examined).
17 per cent. also had cutaneous syphilis.
13 per cent. also had neurosyphilis.
10 per cent. also had visceral syphilis.

Of 21 patients with visceral syphilis (splenic, hepatic or gastric):
14 per cent. also had cardio-vascular syphilis (of 7 specially examined).
25 per cent. also had neurosyphilis.
14 per cent. also had osseous syphilis.

Of 17 patients with cutaneous and mucous membrane syphilis:
50 per cent. also had cardio-vascular syphilis (of 6 specially examined).
6 per cent. also had neurosyphilis.
30 per cent. also had osseous syphilis.

Of 10 patients with heredosyphilis:
33 per cent. also had cardio-vascular syphilis (only 3 specially examined).
20 per cent. also had neurosyphilis.
20 per cent. also had osseous syphilis.
40 per cent. also had interstitial keratitis.
30 per cent. also had eighth nerve deafness.

From the standpoint of diagnosis, the practical importance of the subject is obvious; from the standpoint of specific therapy, the subject is of scarcely less importance because the untoward effects of the drugs upon certain organs are to be remembered in connection with an appraisal or estimate of the factors influencing the selection and administration of drugs, tolerance for them, dangers attending their use, especially the possibility of producing Herxheimer reactions and paradoxical results, etc., as well as the special therapeutic measures sometimes indicated for disease of certain organs. The subject of dissemination of lesions and their detection is one of primary importance, therefore, as the first step in outlining the treatment of cases of chronic syphilis and not only specific treatment but general treatment and hygiene as well. For example, to plan a vigorous course of intravenous injections of arsphenamin or one of its substitutes for a case of neurosyphilis while ignoring or overlooking important lesions in the cardio-vascular system may be an error by reason of accidents to the latter; likewise, vigorous arsenical treatment of an osseous lesion when the possibility of dangerous neural Herxheimer reactions is overlooked.

Advisability of Thorough Examinations in the Diagnosis of Chronic Syphilis.—Because of this possibility of disseminated lesions in chronic syphilis it may not be readily possible for any one specialist to properly gauge a patient as a whole; the opthalmologist, for example, is naturally mostly interested in the eye lesions and may entirely overlook lesions in the cardiovascular system, liver and other organs; complete diagnosis may be too much for any one physician and frequently requires the coöperation of several specialists and particularly of the opthalmologist, neurologist, roentgenologist and serologist. The complete diagnosis of chronic syphilis is, therefore, a matter of considerable importance and under ideal conditions should provide for any or all of the following examinations of each patient:

1. A very careful personal, family and social history.
2. Blood Wassermann tests by a reliable method combining in greatest degree the maximum of sensitiveness consistent with specificity.
3. A complete examination of the spinal fluid in some cases and especially those whose history or physical condition is suggestive of chronic syphilis but whole blood Wassermann tests yield negative reactions. These examinations should be for pressure, total cells, protein, Wassermann and colloidal gold reactions. In some cases a provocative Wassermann test may be indicated.
4. A complete examination of the urine and especially for albumin, casts, pus and red blood corpuscles.
5. A neurologic examination.
6. An examination of eye, ear, nose and throat.
7. An examination of the skin, mucous membranes, periosteum and bones.
8. A cardiovascular examination, including the physical findings, blood pressure and in some cases, an electrocardiographic study.
9. An examination of the abdominal viscera and especially of the stomach, intestines, liver and kidneys.
10. A roentgenologic study and especially of the heart and aorta.
11. A therapeutic test in some cases.
12. An examination for foci of infection as necrotic teeth, impacted roots, apical abscesses, enlarged tonsils, infected sinuses, etc. The removal or correction of these is advisable in the way of preparatory treatment to reduce to a minimum the chances of ill effects from the administration of arsphenamin and its substitutes and especially dermatitis and allied reactions.

In succeeding chapters, I will mention the chief pathological changes in chronic syphilis of the important vital organs although the matter of diagnosis is not included in a manual of this kind devoted to the subject of treatment; but in many cases the application of comparatively simple tests and examinations may suffice to show that an organ or system is not involved to the extent of warranting serious consideration from the standpoint of treatment. In other words the well-trained physician may handle many cases without assistance, provided he recognizes the advisability and frequently the necessity for a complete survey of this kind before outlining a plan of treatment.

Limitations of the Therapeutic Test.—The therapeutic test is not now employed as frequently as formerly, but since it is of much more importance in the diagnosis of chronic than of early syphilis, it is well to recall its limitations and even dangers. Stokes[2] has recently summarized this subject as follows: "Therapeutic tests in general have meaning only when the patient presents a definite, and, as far as possible, a visible pathologic lesion, on which quantitative estimates of improvement can be made. Mere gain in weight, disappearance of indefinite pains, malaise or nondescript subjective symptoms are usually meaningless. Iodid therapeutic tests, popular with the departing generation, are untrustworthy. So are arsphenamin therapeutic tests. I have made, and seen made, erroneous diagnoses of gumma of the lymph nodes with partial positive Wassermann tests. I have seen tuberculous keratitis confused with syphilitic keratitis, lupus vulgaris, erythematous lupus, sarcoids, and tuberculids make striking improvement under arsphen-

amin. Mercury is probably more nearly immune from such nonspecific effects than either iodids or arsphenamin, although it is well to recall its action in lichen planus, and in occasional cases of sporotrichosis. Carcinoma of the stomach makes false responses to arsphenamin alone and sometimes in combination with mercury. One of the reasons a noted neurologist gave for objecting to the dermatologist as a syphilographer was his ignorance of the false positive therapeutic test for syphilis in multiple sclerosis." Stokes also states that he agrees with Herxheimer and Altman[3] that arsphenamin may produce pseudo-reactions in tuberculous processes analogous to the reactions produced by large doses of non-specific proteins and occasionally create a deceptive effect.

The mere fact that a particular lesion shows some regression under specific treatment does not necessarily mean, therefore, that it is syphilitic. Furthermore, the institution of a therapeutic test by the injection of arsphenamin or one of its substitutes may result in the production of a Herxheimer reaction of the optic or auditory nerves, in the heart, a laryngeal ulcer, etc., the dangers and possibilities of which should be estimated and kept in mind.

Pathology of Chronic Syphilis in Relation to Specific Therapy.—This subject has already been discussed at length in Chapter XXXVIII, but it may be well to recall here that the chronic pathological tissue changes produced by *Sp. pallida* constitute the chief reason why the treatment of chronic syphilis is an almost entirely different problem from the treatment of early syphilis and why individualism in treatment is so much more necessary and important than in the latter. The chronic pathological tissue changes of syphilis may influence the efficacy of treatment, the choice and administration of antisyphilitic drugs, tolerance and untoward toxic effects of the drugs and finally therapeutic expectancy and prognosis.

The chief tissue changes in relation to treatment are those occurring in the blood-vessels consisting of mesarteritis and perivascular infiltrations and fibrosis which may hinder the escape of medicaments from the blood stream and may actually amount to an obliterating endarteritis. Even if these changes are not marked the parasites are apt to be well surrounded with plasma cells, lymphocytes and scar tissue which renders difficult the access of spirocheticides. It is largely for these reasons that the iodids constitute valuable remedies in chronic syphilis by reason of their influence upon such organized and unorganized exudates and for the relief of pain probably by release of pressure upon sensory nerves; also why mercury and possibly bismuth, constitute valuable remedies because the former at least tends to gradually penetrate and accumulate in such lesions until spirocheticidal concentrations are reached; also why small doses of the organic arsenicals frequently repeated are usually better than fewer but larger doses, because a better opportunity is afforded for the penetration of lesions when arsenic is available over relatively long periods of time and, finally, why intramuscular injections of the organic arsenicals are sometimes better than intravenous injections, by reason of slower absorption, slower elimination and consequently more prolonged contact of the spirocheticide with the foci of infection.

Tolerance for drugs and untoward effects following their administration are largely influenced by possible pathological changes due to syphilis and other diseases involving the matter of their metabolism and elimination. The kidneys may be defective, not so much because of syphilis, but by reason of chronic interstitial and parenchymatous changes of other origin; the liver may be defective not only because of syphilitic infection but also by reason of infection of the gall-tracts with streptococci or other organisms. Dysfunction of these organs as well as of the skin, heart, etc., due to chronic syphilis

or other diseases may very materially influence the toxicity or organotropic activity of antisyphilitic drugs or at least, influence their selection and manner of administration in an important manner. Finally, the prolonged administration of the drugs themselves and especially arsphenamin and its substitutes, mercury and bismuth, may result in the production of added pathological tissue changes as discussed in Chapters XLVI, LIII and LV.

The additional tissue changes of chronic syphilis are largely concerned with those produced in the cells of the organ or tissue involved as a result of toxic substances produced by *Sp. pallida* or secondary to the vascular changes and production of inflammatory exudates. These changes are multitudinous and especially in the brain and spinal cord, but it is hardly worth while to mention here the minute changes because they do not primarily influence treatment. They do, however, bear an important relation to therapeutic expectancy and prognosis. For example, in the treatment of tabes dorsalis the knee-jerks and a normal gait may not be restored because antisyphilitic drugs cannot remove scar tissue; likewise sclerosis of the aortic valve and of the coronary arteries may not be removed. What treatment aims to accomplish is to prevent further tissue changes by *Sp. pallida* while hoping for at least some symptomatic improvement. Indeed a new set of symptoms and lesions may make their appearance as a result of healing since the further laying down of fibrous tissue may follow the sterilization of foci; such, for example, may be ascites and other evidences of cirrhosis of the liver following the effectual treatment of syphilitic hepatitis and constituting a therapeutic paradox as pointed out recently by Wile;[4] Stokes states that cicatricial stenosis of the oesphagus and rectum, even of the hour-glass stomach, may occur and he is inclined to believe with Hubert[5] that, so far as the heart is concerned, acute myocardial ischemia, anginal attacks of increased severity, even the development of certain physical signs of aortitis and aneurysm, may be the results of too energetic treatment and too rapid healing.

Principles of the Treatment of Chronic Syphilis.—It is apparent, therefore, from this brief review of the pathology of chronic syphilis in relation to treatment, that many factors may operate against the attainment of successful results. In addition to the pathological tissue changes produced in vital organs by *Sp. pallida*, we may have those so-called physiological changes due to advancing years and the effects of any intercurrent infections to which syphilitics are as much subject as non-syphilitic individuals. We also run the danger of breaking up the immunity equilibrium of latency in our anxiety to cure, without being able to successfully combat an increased activity of aroused spirochetes and resulting in the production of a Herxheimer reaction.

We should, therefore, carefully appraise each case before planning a therapeutic program based upon the age of the patient, an estimate of life expectancy and the influence of handicaps as tuberculosis, chronic nephritis, arterial hypertension, etc.; also an estimate of probable tolerance for antisyphilitic drugs and the probable response to treatment on the part of the most important organs involved.

Our therapeutic program should include a thorough examination to detect the weakest points and most dangerous lesions, with a view to finding all the types of involvement in each case. This may enable us to regulate the life of the patient in such manner as to relieve strain on the weakest points and to conduct treatment in such manner that an early involvement may be checked from becoming later a prominent lesion.

We should use every available means for improving the general condition and to protect against the ill effects of the drugs administered with a correction of those defects likely to produce toxic drug reactions or favor spirochetic

activity. I have in mind such matters as proper attention to the bowels and kidneys, estimating the functional capacity of the latter not only by frequent examinations of the urine, but also by occasional estimations of blood urea and total creatinine; oral hygiene and the extirpation of focal infections; the avoidance of dietary errors, excessive smoking, alcoholism, over-work and worry, sexual excesses, etc. In other words as stated by Stokes, our method should be a general overhauling job rather than a patching job. As he has so well stated, it is well to "protect patients from the small but cumulative insults which too often keep them in a substandard condition under intensive treatment, even though the individual by-effect may not become conspicuous in itself. In late syphilis, in which the margin between treatment requirements and treatment tolerance is often very small, such protective measures may make all the difference between success and failure."

From the standpoint of specific therapy, it is more difficult to adhere to any set or fixed plan of treatment of chronic syphilis than for early syphilis, although it is well and advisable to have a scheme of treatment fixed in mind as a model of routine therapy subject to modification for individual cases, rather than to carry the plan of individualization too far. This is especially true in clinic practice where physicians may be required to manage large numbers of patients in a comparatively brief time; it insures more thorough treatment of these as well as of patients in private practice.

Mercury and the iodids should certainly play a very important part in treatment; likewise bismuth as a substitute for mercury. But the organic arsenicals are likewise of great aid and importance although the intensive methods are required only for the rapid healing of open lesions and the general principle being one of less intensive but more prolonged specific treatment than in the case of early syphilis in which the total dosage of specifics equals and possibly exceeds the amounts given in the latter. Moderation in dosage is imperative and especially in the treatment of chronic cardio-vascular syphilis; treatment should be based largely upon close and frequent observation and examination at intervals for many years and even for the balance of life in order to direct treatment toward the conservation of vital organs essential to life, even though involvement of physiologically unimportant tissues must be overlooked. In other words I thoroughly agree with Stokes who has written that "the essence of the modern treatment of syphilis is system and observation, and in the end those of the modern treatment technics which best perfect the *observational* aspect, will show the lowest ultimate mortality." Even after symptoms and lesions disappear, observation is required for many years and even for the balance of life with as much follow up treatment at least as given to early cases of syphilis, if tolerance permits.

Criteria of Successful Treatment of Chronic Syphilis.—Finally what may be reasonably expected of the modern treatment of chronic syphilis? Of course, our aim is complete cure without producing irreparable or dangerous reactions by the drugs employed. We do not yet know what percentage of cases are being radically cured; probably it is but a small number of the total treated under the best of conditions, but arrest of the disease is apparently attainable in at least 80 to 90 per cent. of cases and symptomatic improvement and life long arrest of progress of the disease may almost amount to the same thing as total extirpation of the infection. Carrying an individual through the fulness of his or her years, with the maximum degree of working capacity and efficiency and the minimum of danger to contacts and society, comes very near to being a successful result and such are obtainable in many cases.

Our patients commonly expect too much. It is not reasonable to expect the removal of the scars or complete arrest in a matter of a few months of an infection that has been progressive for years. It is of little importance that knee-jerks and gait are not restored, that an Argyll-Robertson pupil persists or a murmur of aortic regurgitation is undiminished. It is important, however, that the further loss of function cease, as progressive disturbances of gait or the development of bladder and rectal incontinence, increasing frequency of anginal attacks, increasing size of the liver and attacks of jaundice, etc., depending upon the organ or organs mostly involved. As previously stated, paradoxical effects may be observed in which a new set of signs and symptoms may appear as a result of healing which are readily enough misinterpreted as signs of progressive syphilis, as, for example, the development of ascites in the course of healing of syphilitc hepatitis, optic atrophy as a syphilitic neuroretinitis subsides, etc. As shown in aneurysms, pulsations may cease as mesaortitis and periortitis subside so that structural or anatomical improvement may not follow, at least immediately, upon the disappearance of signs and symptoms, since only time and much of it, can be expected to bring about regressive changes in organs like the cardio-vascular, renal and gastro-intestinal systems which cannot enjoy functional rest for recuperation after infection has been extirpated or controlled. On the other hand the rapid improvement in general nutrition, weight and general health as well as functional improvements and especially of the eye and ear as unorganized exudates are absorbed, sometimes exceeds all expectations and lends great encouragement in general to the treatment of chronic syphilis.

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CHAPTER LXXV

SPECIFIC TREATMENT OF VISCERAL SYPHILIS

Having thus reviewed in the preceeding chapter the very important subject of the principles underlying and governing the general and specific treatment of chronic syphilis, the treatment of special types may be now considered. I realize the folly of even attempting to formulate anything approaching fixed or standard methods, but yet, as stated in the preceeding chapter, it is distinctly serviceable to have these in mind, subject to alteration to meet the indications and requirements of individual cases. At least, this insures to a better degree against undertreatment and has greatly aided my own work; I am, therefore, giving in this chapter methods for the treatment of different types of chronic syphilis other than of the central nervous system, which I hope will prove as helpful to others as they have to me.

Specific Treatment of Cardiovascular Syphilis.—Syphilitic infection of the cardiovascular system, and especially of the heart and ascending aorta, is apparently the most common type of chronic visceral syphilis. Indeed, this

is so true that the syphilologist should never neglect to examine or have examined the cardiovascular stripe in all cases of chronic syphilis and the internist should never forget the possible syphilitic basis for many of the chronic diseases of these organs. Owing to the ease and frequency with which invasion and infection of the meninges, brain and cord are detected by examinations of the cerebrospinal fluid, we ordinarily have the impression that infection of these tissues with *Sp. pallida* is the most frequent of visceral involvements; but the incidence of cardiovascular syphilis is higher, both clinically and especially pathologically, as determined by the high percentage of macroscopic and microscopic lesions found after death even among syphilitics presenting no particular clinical evidences of involvement during life. Indeed, it is highly probable that almost all cases of chronic syphilis of many years standing have some infection of the cardiovascular system and especially of the heart muscle and ascending portion of the aorta. This is so because of the inherent or natural tendency and predilection of *Sp. pallida* for localization in the heart muscle, the mesial coat and perivascular lymph spaces of blood-vessels, and particularly of the arteries, and because the heart, aorta and arteries of other functionally active organs like the brain and meninges are so frequently subjected to strain and trauma.

Cardiovascular Lesions of Chronic Syphilis.—A rather large literature has accumulated upon this subject, since Morgagni and other early writers first called attention to the syphilitic nature of aneurysms. Within recent years the studies of Brooks,[1] Howard,[2] Symmers,[3] Warthin,[4] Levison,[5] Babcock,[6] Tyler,[7] Hubert,[8] McKensie,[9] and numerous others have greatly aided in drawing attention to the importance of this subject with improved means for clinical diagnosis during life and detection after death. My pathological classification of these lesions is as follows:

(*a*) Syphilitic pericarditis and epicarditis { Diffuse / Localized (gumma)

(*b*) Syphilitic myocarditis { Parenchymatous / Interstitial / Diffuse (usual)

(*c*) Syphilitic endocarditis—usually of aortic valves with regurgitation

(*d*) Syphilitic sclerosis of coronary arteries

(*e*) Syphilitic aortitis { Without dilatation / With fusiform dilatation / With aneurysm

(*f*) Syphilitic arteritis of peripheral vessels { General arteriosclerosis with or without hypertension. / Local arteriosclerosis. / Aneurysms.

(*g*) Syphilitic phlebitis of peripheral veins { General / Local with thrombosis

In a treatise of this kind detailed descriptions of the gross and pathological tissue changes are not demanded, except insofar as these bear a relation to treatment. While detailed accounts of the histological changes are lengthy and complicated, the subject after all can be reduced to rather simple terms. In other words, the pathological changes produced by *Sp. pallida* in the cardiovascular system are similar in every way to those produced in other organs, namely, (*a*) the production of inflammatory exudates of plasma cells and small lymphocytes, especially around small vessels and lymphatics, except in cases of extreme latency when these may be almost absent; (*b*) the production of albuminous, hyaline, fatty or other degenerations of the parenchymatous cells as a result of spirochete toxins and particularly vascular occlusions, and (*c*) the production of fibrous tissue, as in all chronic inflammations.

As would be expected, that portion of the heart and aorta subject to most strain (the anterior wall of the left ventricle and first portion of the aorta) are

most likely to show involvement. Aortic valvulitis, sclerosis and aneurysms of the ascending aorta are the most frequent vascular lesions; indeed, aneurysms of the aorta and especially of its ascending and arching portions, is so frequently syphilitic that all should be regarded as syphilitic until proven otherwise. While sclerosis of the coronary arteries is of frequent occurrence, yet angina pectoris is only exceptionally of syphilitic origin and heart block is but rarely due to syphilis, although Gaucher[10] and others claim that angina pectoris is frequently of syphilitic origin. However, writers on this subject have not always clearly distinguished between precordial pain due to aortitis and stretching of this vessel and true angina pectoris. Of the peripheral vessels, involvement of the cerebral arteries, and especially of the middle cerebral artery and its branches, is best known because of the frequency of aneurysms, thrombosis and rupture of these vessels with the production of hemiplegia due to the frequency of their involvement and poor support by the cerebrospinal fluid and soft consistency of the brain tissue. Hypertension is so frequently due to chronic cardiovascular syphilis, as shown by Audbry and Nanta[11] and others, that this origin is always worthy of serious consideration and study.

General Treatment of Cardiovascular Syphilis.—This varies greatly according to the requirements of individual cases. But all require warning against sudden or prolonged strain, as in laborious work or strenuous exercise. In many cases, probably in the majority, nothing more is required in the way of general treatment than additional advice against excessive smoking, the excessive use of alcohol and coffee, excessive venereal indulgence and all other forms of high tension activity. The anxiety neurosis so often exhibited by these patients must be combated, as it is sometimes worse in its effects than the infection itself; the patient must be educated to a matter-of-fact attitude and a uniform level of physical and mental strain, with a minimum of periods of sudden exertion, anger, worry, etc. If hypertension exists these and additional measures should be taken to reduce it; as a rule, cases with hypertrophied heart with good compensation do well under treatment and especially small doses of neoarsphenamin by intravenous injection. The key-note is the prevention of sudden or long sustained strain, not only because present lesions may "give away," but also because new areas of infection may be produced or latent foci stimulated into activity.

In severe cases of cardiac decompensation and dilatation, rest in bed with the administration of digitalis, strophanthus, etc., dietary restrictions to prevent increase of weight and to lessen the burden of nitrogenous elimination by the kidneys, dieuretics and other special measures may be required.

Specific Treatment of Cardiovascular Syphilis.—Specific medication is possible and advisable in the great majority of cases. However, as stated by Mock[12] and others, marked prostration, severe and frequent angina and severe decompensation and dilatation are bad risks or positive contra-indications, unless rest in bed combined with judicious general treatment restores the patient to such a state that mild antisyphilitic treatment may be cautiously undertaken.

It is important, however, to institute specific treatment in a mild and progressive manner, rather than administer large doses of arsphenamin or its substitutes by intravenous injection in order to prevent Herxheimer reactions and the untoward effects of the drugs or the excitement of their administration upon blood-pressure and capillaries in general. Most writers are in favor of beginning with mercury and iodids or, at least, depending largely upon prolonged treatment with these, as Dix,[13] Babcock,[14] Etienne,[15] Wile,[16] Groedel and Hubert,[17] Stokes[18] and others. While Vau-

quer, Laubry and Donzelot[19] treat these cases rather energetically with the arsenicals and mercury, the majority of other writers like Mock, Howard[20] Kothny and Müller-Deuham,[21] and Cumston[22] have preferred prolonged treatment with very small doses of arsphenamin and its substitutes, and especially neoarsphenamin, and this appears to be the keynote of success in the specific treatment of these cases, at least, cases far enough advanced to influence the toxic effects of the drugs. Harrison[23] has observed good results in the treatment of myocarditis following deep subcutaneous or intramuscular injections of sulfarsenol (sulpharsphenamin) in dose of 0.3 to 0.48 gm. combined with the administration of mercury and iodid. Herxheimer reactions and cardiac strain from large doses of antiluetic drugs, and especially the arsenicals, may produce an increase of pain, pulmonary edema and anasarca, increased expansile pulsations of aneurysms or rupture of the latter, and such accidents are avoidable by beginning treatment in a mild manner with mercury or very small doses of neoarsphenamin (0.05 to 0.1 gm.) intravenously. Rosin[24] has expressed an opinion in favor of bismuth and, unquestionably, good results are to be obtained with intramuscular injections as a substitute for mercury. The iodids are especially good for the pain of aortitis and coronary sclerosis, but large doses by intravenous injection, as advised by Hoppe-Seyler[25] are unnecessary. Schottmüller[26] has advised weekly intravenous injections of 0.3 to 0.45 gm. of neoarsphenamin until 5 grams have been given, combined with intramuscular injections of calomel or salicylate of mercury in dose of 1 to 1½ grains once or twice a week or intravenous injections of cyarsol or novasurol in separate syringes. Potassium iodid is given for one to two months after each course and two complete courses are given each year for at least three years. In severe cases monthly injections of neoarsphenamin are advised.

With these examples of variation in present methods I may now describe my method for the *specific treatment* of the average case of chronic cardiovascular syphilis under 65 years of age in good general condition, although the urine may show a faint trace of albumin with few hyaline casts:

1. Two months of mercury by inunction every other day in dose of one dram of 50-per cent. mercurial or 85-per cent. calomel ointment with daily doses of potassium iodid by ingestion in amounts of 20 to 45 grains. If inunctions cannot be given, epifascial injections of salicylate or succinimid of mercury in dose of 1 grain or intramuscular injections of potassium tartrobismuthate in dose of 0.1 to 0.2 gm., are given once a week for eight doses.

2. The administration of arsenic is now begun in dose of 0.1 gm. of neoarsphenamin twice a week for three months (24 injections). If well borne, the dose is gradually raised and the whole course generally averages a total of about 5 gm. Arsphenamin is not given because of the greater chances of untoward effects. Sulpharsphenamin may be substituted by epifascial injection giving 0.2 gm. every 5 to 7 days and gradually increasing to 0.4 gm. per dose if well borne so that a total of about 4 gm. are given in the course of twelve weeks. At present I frequently alternate intravenous injections of neoarsphenamin with epifascial injections of sulpharsphenamin. Potassium or sodium iodid is given throughout in well-tolerated doses.

3. The urine is examined routinely at frequent intervals.

4. Immediately following the arsenical course 1 grain of protiodid of mercury a day in divided doses or 1 grain of mercury with chalk combined with 1 grain of Dover's powder, three times a day, are prescribed for a period of two months, the patient reporting at intervals for examination.

5. This is followed by a rest of two months and a quantitative Wassermann reaction. Sometimes in cases of aortitis associated with pain, it is not

advisable to stop specific treatment altogether but to continue with weekly injections of neoarsphenamin in dose of 0.2 to 0.4 gm. or intramuscular injections of potassium tartrobismuthate in dose of 0.2 gm. throughout this period.

6. The course described in paragraph 2 is now repeated, which finishes the first year of treatment.

During this time there has usually occurred great relief from pain, pulsations, etc., along with arrest of the lesions, some regression and especially of early fusiform dilatations of the aorta, and frequently considerable diminution of the degree of complement fixation sometimes amounting to a complete reversal to negative. But specific treatment should not stop; at least one or two courses should be given during the second and third years and in some cases for longer periods, depending upon circumstances, of the following:

7. For two months either (*a*) three inunctions of mercury per week with weekly intravenous injections of neoarsphenamin (0.2 to 0.4 gm.) or weekly epifascial injections of 0.2 gm. sulpharsphenamin or alternate injections of these; or (*b*) alternate weekly intravenous injections of 0.2 to 0.4 gm. neoarsphenamin combined in the same syringe with 10 c.c. of 2-per cent. flumerin (0.2 gm.) or 1:1000 mercurophen (0.01 gm. or $\frac{1}{6}$th grain) with intramuscular injections of potassium tartrobismuthate in dose of 0.2 gm. Potassium iodid is given with either course by ingestion in dose of 40 to 60 grains daily if well tolerated.

This is followed by two months of rest and two months of protiodid of mercury or mercury with chalk by mouth. The course requires six months and two may be given the second year and one the third year.

8. But it is doubtful if specific treatment should be stopped entirely after this time, even though Wassermann tests yield negative reactions. It is generally advisable for these patients to report regularly twice a year for examination and to take at least one course of treatment per year of either of the two courses described in paragraph 7 or at least two or three months of daily inunctions of mercurial or calomel ointments with potassium iodid by mouth. If inunctions cannot be given, intramuscular injections of potassium tartrobismuthate in dose of 0.2 gm. or salicylate of mercury in $\frac{1}{2}$ to 1 grain, for eight weekly injections, may be administered. If these are not required, at least three months of mercury by ingestion per year should be advised as a precautionary measure.

Prognosis in Cardiovascular Syphilis.—The prognosis is, of course, subject to great variation depending upon the location of the lesions, their duration and severity before treatment is instituted. In severe cases the duration of life is ordinarily from two to five years. If specific treatment is undertaken early and the patient is willing to follow advice as to the manner of living, the prognosis is ordinarily quite favorable and many, probably the majority, of patients can be carried along for many years and even to the usual span of life. According to Levinson[27] involvements of the aortic valves and coronary arteries are always serious; Keidel and Kemp[28] state that the prognosis in uncomplicated aortitis is ordinarily quite good when well treated, bearing a relation to the amount, regularity and duration of specific medication. Special care must be exercised in treatment when aneurysms are present to guard against the effects of Herxheimer reactions and toxic reactions. The symptoms are generally relieved while the lesion remains stationary for long periods of time even though it may not recede but remain as early fusiform dilatations. All of these results are worth while and may amount to a symptomatic cure even though total extermination of the infection may not be possible and very probably is of only exceptional occurrence.

Specific Treatment of Syphilis of the Liver. *Mechanism of Infection and the Pathology of Hepatic Syphilis in Relation to Specific Therapy.*—Owing to the large size, rich blood supply and functional activity of the liver, it is to be expected that this organ will be frequently infected with *Sp. pallida.* Symmers,[29] for example, found hepatic lesions in 105 of a series of 314 post-mortem examinations of syphilitics and this incidence was based largely upon macroscopic evidences; doubtless histological examinations would have disclosed a higher incidence. But clinically the incidence is not nearly as high, since infection of the liver may be present without the production of signs and symptoms; it is surprising to note how much damage of the liver may be found at necropsies in individuals presenting no particular evidences of hepatic disturbances during life. There is no evidence indicating the existence of any specific hepatotropic strains of the parasites, but doubtless the subject of previous trauma exercises the same favoring or predisposing influence upon the localization of the spirochetes in this organ as is true of the cardiovascular and other organs. Probably the excessive use of alcohol is important in relation to this matter of predisposing tissue injury, but hepatic syphilis has not been especially observed in relation to chronic alcoholism, as have been some non-syphilitic types of hepatic cirrhosis.

Very probably the liver is most frequently invaded and infected during the initial period of spirochetemia of early syphilis, as occurs in the central nervous system, although we do not have the direct evidence of this, as is furnished by spinal fluid examinations; clinically, not more than 1 per cent. of syphilitics show evidences of the infection at this time in the form of jaundice. But in my opinion the liver may be infected at subsequent intervals by way of the vascular or lymphatic systems.

In vascular infections, and these are doubtless the more frequent, the spirochetes are lodged in the walls of the capillaries and in the perivascular spaces. Pathologically we may recognize a vascular type of hepatic syphilis analogous to the vascular types of neurosyphilis, but clinically this is not detectable except in those rare cases of aneurysm of the hepatic artery and thromboses of syphilized veins. How and why the spirochetes in one case invade the parenchyma in a widespread manner producing diffuse hepatitis in some cases and remain localized in others, cannot be stated, just as we are at a loss to explain why parenchymatous syphilis of the brain (paresis) occurs in some and not in all cases of neurosyphilis.

After lodgement in the liver, acute changes may be produced in the liver cells and these in turn involve the bile ducts; or both, and especially the latter, may be involved by the perivascular exudates. Later fibrous tissue is laid down with another train of secondary changes due to atrophic compression of the parenchyma and bile ducts, as well as loss of nutrition by reason of capillary fibrosis. It is no wonder, therefore, that pathologists have published elaborate accounts of the gross and microscopical changes in hepatic syphilis, because there are so many opportunities for variations according to the degree and duration of involvement, just as elaborate classifications of the lesions of neurosyphilis, and especially of the brain, have been evolved by systematic histological studies. But from the clinical standpoint, and especially in relation to the specific therapy of hepatic syphilis, a relatively simple clinico-pathological classification suffices, and that given herewith has been found adequate and useful:

A. Acute syphilitic hepatitis—diffuse
- Mild icterus
- Severe icterus (icterus gravis syphiliticus or icterus syphiliticus præcox)

B. Chronic syphilitic hepatitis
- Diffuse
- Localized
  - Gummas, including perihepatitis
  - Cicatrices ("hepar lobatum")
- Combined diffuse and localized including amyloid infiltrations.

This classification does not include, therefore, vascular hepatic syphilis or syphilis of the biliary ducts or gall-bladder, because the former is not usually clinically recognizable and the latter do not appear to be primary syphilitic involvements, although the bile ducts are practically always and the gall-bladder sometimes, involved in the course of diffuse and localized syphilis of the liver parenchyma.

From the standpoint of specific therapy hepatic syphilis sometimes presents unusual difficulties because of interference with the metabolism and elimination of specific remedies and especially of arsphenamin and its substitutes. However, when we are reasonably sure of the diagnosis of syphilis and the syphilitic origin of a jaundice, bold specific therapy is indicated and yields gratifying therapeutic results. But in not a few instances the jaundice and other symptoms are not primarily due to syphilis but to a superimposed streptococcus, colon bacillus or other type of infection of the gall-bladder and ducts, and such cases do not improve under specific therapy and indeed, sometimes display a marked degree of intolerance for the organic arsenicals and sometimes for mercury as well. The matter of differential diagnosis of jaundice in syphilis is, therefore, one of considerable importance, as it may be due to syphilis or to other infection, to arsphenamin or other arsenical, or to a combination of these, as discussed more at length in Chapter LI, on the subject of arsenical jaundice. The presence of jaundice, a subicteric state or a history of jaundice always demands unusual care in the outlining of treatment for syphilis; each case must be handled individually, and specific treatment initiated and continued with great care to avoid added injury of the liver, if syphilitic hepatitis is the minor change, or to check the syphilitic process, if this infection is the major one. Naturally when a syphilitic patient presents himself with jaundice we believe that it may be due to syphilis; if he develops jaundice during treatment and especially with arsphenamin, we conclude that it is arsenical jaundice. We may be wrong in both instances, since the jaundice may be due to some other type of infection, carcinoma, etc. Therefore hepatic involvements in syphilis are among the most difficult of diagnosis and sometimes difficult of therapeutic management.

Furthermore, as so clearly described by Wile,[30] the cure of syphilitic hepatitis may be followed by ascites and other symptoms due to the contraction of fibrous tissues following the absorption of syphilitic exudates and gummas; in other words, a paradoxical result may be observed in that the eradication of syphilitic infection may be followed by a train of sequelæ worse than the syphilitic hepatitis. Ascites, however, is inevitable and would have followed anyhow in the absence of treatment; but Wile has properly emphasized the possibility of its early development as the result of specific treatment and as one of the peculiar difficulties that may be encountered in the specific treatment of syphilitic hepatitis.

Insofar as the choice of antisyphilitic drugs are concerned, mercury, bismuth and the iodids are probably better borne than the organic arsenicals, and of the latter, neoarsphenamin is to be preferred to arsphenamin if one may draw this conclusion on the basis of comparative histological studies of the effects of the two compounds upon liver cells. Clinical experience also indicates that arsphenamin is more likely to produce hepatitis than neoarsphenamin, but either drug may be employed if proper attention is given to the matter of dosage.

Specific Treatment of the Hepatitis of Early Syphilis.—This refers to the hepatitis occurring in the late primary and secondary stages of early syphilis. Various explanations have been offered to account for the occurrence of the jaundice. In my opinion it may be due to obstruction of the common bile duct as a result of catarrhal duodenitis, which in turn may be due to a syphi-

litic exanthem of the mucosa; or, it may be the result of constriction of bile ducts by the pressure from edema and cellular exudates in the perivascular spaces invaded by *Sp. pallida*, in addition to involvement of the liver cells at the periphery of the lobules. At any rate, it rarely offers any difficulties in treatment and specific medication may be along the lines described in Chapter LXXII for the treatment of early syphilis, except that preference is to be given to neoarsphenamin rather than to arsphenamin; the diet may be especially of carbohydrates, because liver cells full of glycogen probably do not take up the heavy metals in case the organic arsenicals and mercury are administered. If, however, the patient gives a history of jaundice preceding the contraction of syphilis and thereby showing that the hepatic disorder is probably of bacterial or other origin, greater care will be required in the treatment in the way of less intensive specific therapy.

Specific Treatment of Severe Syphilitic Hepatitis (Icterus Gravis Syphiliticus).—This disease also occurs in early syphilis and runs the course of acute yellow atrophy of the liver. It is apparently an extreme degree of hepatitis due to profound and overwhelming infection of the liver with *Sp. pallida*. How and why the disease occurs is unknown and the condition has no analogue in any other organ, that is to say, no other organ of the body is known to be subject to such a similar degree of profound acute degeneration in the early stages of syphilis. There must be a predisposing change or cause; pregnancy has been associated in some cases. Fortunately the disease is rare and most cases have ended fatally. Wile, however, records the recovery of a case under vigorous mercurial and iodid treatment, and these two drugs appear to be the remedies of choice, since the arsenicals are contraindicated on account of the extreme hepatitis, which may interfere with the elimination of the toxic arsenical metabolites. The diet should be principally of carbohydrates.

Treatment of Chronic Syphilitic Hepatitis.—In the treatment of chronic hepatitis it is my custom first to estimate the functional capacity of the organ by the phenoltetrachlorphthalein and bilirubin tests described on page 705. If the former is eliminated normally and the bilirubin content of the blood is within physiological limits, as they may be and usually are in cases of localized gummas of the liver, the case may be treated in much the same manner as described for the treatment of chronic syphilis in general in Chapter LXXIII, except that special emphasis is to be placed upon large doses of the iodids, like 60 to 90 grains per day by ingestion, if sufficiently well tolerated. If however, the functional tests, the changes in the topography of the liver and the degree of jaundice indicate a severer involvement of the parenchyma due to numerous localized gummas or, as is usual, a combination of diffuse and localized hepatitis, it may be advisable and necessary to change this plan of treatment to consist mostly of mercury or bismuth and large doses of the iodids. Not infrequently the first course of mercury or bismuth is sufficient for clearing up the jaundice so that a course of neoarsphenamin may follow as described; but it is well to use small doses at first (about 0.2 gm.) and cautiously increase them if good tolerance is observed. In such cases, however, epifascial injections of sulpharsphenamin may be better, since absorption is slower with probably less demand upon the eliminative functions of the liver. The diet should be principally of carbohydrates during the icteric stages. In my experience these cases do not offer as much difficulty in the way of specific medication as the following group.

Cases of Syphilis Developing Jaundice during Specific Treatment.—These are the most difficult cases of all and especially from the standpoint of diagnosis of the nature of the jaundice. If this is due to syphilis as a result of a

Herxheimer reaction, that is, an increase of biliary obstruction from increased spirochetic activity, the indications are to push treatment with neoarsphenamin or sulpharsphenamin, as advised by Milian, for the purpose of checking and reducing the inflammatory changes produced by *Sp. pallida.* Such is usually the case when jaundice develops within a week or two after the institution of specific treatment, and especially after the first one or two injections of an arsenical compound. If, however, jaundice develops later in treatment it cannot be considered a hepatic Herxheimer reaction but rather one of the following: (*a*) the result of a superimposed bacterial infection producing obstructive jaundice by catarrhal duodenitis; or (*b*) the lighting up of a previously existing infection of the gall-bladder and biliary system; or (*c*) an arsenical hepatitis; or (*d*) obstruction of the biliary ducts by the products of healing after the manner of Wile's "paradoxical" effects, or (*e*) a coexisting cancer of the head of the pancreas involving the bile ducts and liver or some other superimposed and unrelated disease.

In each case it is advisable to stop specific treatment for the time being. In *a* and *b* special therapeutic measures may be necessary and in *b* the biliary drainage method of Lyon[31] is worthy of consideration as a means for diagnosis and treatment. With the subsidence of the jaundice and associated symptoms, specific treatment may be resumed and especially with mercury or bismuth and the iodids, since one is usually and naturally a little afraid of the arsenicals. If all goes well, however, the latter may be gradually administered and preferably in the form of intramuscular injections of sulpharsphenamin followed later by small, ascending intravenous injections of neoarsphenamin. In the treatment of the *d* kind of case, specific treatment should be continued in the form of large doses of the iodids and active mercurialization, while the ascites is relieved from time to time by tapping as required. As stated above, it is also a good plan to adopt a diet principally of carbohydrates.

Specific Treatment of Syphilis of the Pancreas.—Since syphilis of the pancreas is frequently accompanied by jaundice, it may be well to briefly discuss its specific treatment at this time although it consists of the usual treatment for chronic syphilis described in Chapter LXXIII with such modifications as may be demanded by the coexistence of jaundice or glycosuria.

From the clinical standpoint, syphilis of the pancreas is rare; from the pathological standpoint, however, it is relatively common, according to the investigations of Warthin[32] and Warthin and Wilson. In other words, it may be present without discernible signs or symptoms during life in the majority of cases. Diabetes mellitus, however, may be a result of syphilis of the pancreas or possibly, syphilitic involvement of the medulla and region of the fourth ventricle. Wile[33] has recently summarized the clinical aspects of syphilis of the pancreas and Rosenbloom[34] has discussed the subject very thoroughly in relation to diabetes, including a study of 131 cases of his own; in about 6 per cent. of this series the diabetes was regarded as due to syphilis, while in an additional 6 per cent., syphilis and diabetes were regarded as existing as independent conditions. In other words, diabetes is not usually due to syphilis; but when the blood Wassermann reaction is positive, one may conclude that the patient is syphilitic in addition to being diabetic. This is true at least, of my new complement-fixation test, which Steinfield and I[35] found does not yield falsely positive reactions in non-syphilitic diabetics. On the other hand a positive reaction by this technic indicates that the patient is syphilitic and according to the studies of Warthin, as well as of Sallis,[36] it is entirely likely that the pancreas may be involved.

The jaundice so frequently accompanying advanced syphilis of the pancreas may be due to pressure on the common bile duct by a gumma of

the head of the pancreas or to an associated syphilitic hepatitis. In either case specific treatment should be along the lines described above in which large doses of the iodids and active mercurialization are especially indicated. Even though the parenchyma of the liver is not involved to the extent of interfering with the metabolism of the organic arsenicals, yet if the expulsion of bile from the ducts and gall-bladder is interfered with, there may occur a reabsorption of the arsenic with toxic effects. As long as jaundice is present, therefore, preference should be given to mercury or bismuth and the iodids.

According to most reported cases of syphilitic diabetes, specific treatment does not usually improve the diabetes, because of the destruction of the islands of Langerhans; in other words, we may put out the fire but cannot rebuild the house, and Nature does not appear to be a much better builder herself in this instance. On the other hand, Paullin and Bowcock[37] and others, have observed marked improvement in diabetes during the course of, and probably as the result of specific therapy, the subject being more fully discussed on page 1053.

Specific Treatment of Syphilis of the Spleen.—Owing to the vascularity of the spleen, it is entirely likely that it is infected with *Sp. pallida* as frequently as the liver, although, owing to its relative insensibility in adults and the absence of symptoms, even when it is manifestly involved, clinical syphilis of the spleen is relatively rare in the acquired disease; in the new-born and older children, however, clinical syphilis of the spleen is quite common, probably because of its greater functional activity at this time as well as because of extra severe infection.

The pathology of syphilitic splenitis is quite similar to that of syphilitic hepatitis, in that we recognize an early diffuse splenitis and a late localized, diffused or combined splenitis. The treatment of the former is that of early syphilis in general, as described in Chapter LXXII, and of the latter, that of chronic syphilis described in Chapter LXXIII.

Specific Treatment of Syphilis of the Gastro-intestinal Organs.—Syphilis may involve any portion of the alimentary tract from the salivary glands, gums and tongue to the rectum. Chancres and gummas of the gums are sometimes observed and syphilitic glossitis is relatively common. Syphilitic gastritis is being recognized more frequently as a clinical entity and syphilis of the rectum has long been recognized. Syphilis of the small intestine, however, is comparatively rare, the subject having been recently reviewed by Wile.[38]

The specific treatment of early and late syphilis of these organs is usually that of syphilis in general. Brocq[39] and others have mentioned calomel injections as especially efficacious in late syphilis of the tongue, either in the form of diffuse sclerotic or localized glossitis. In ulcerating gummatous syphilis of the esophagus, pylorus and rectum, however, healing under specific medication may result in strictures, very much as described by Wile in the case of healing of syphilitic hepatitis, producing constriction of the blood and bile channels with ascitis and jaundice. In syphilis of the stomach, the liver is likewise most likely involved and these cases are sometimes difficult to manage from the standpoint of specific therapy. Mercury by inunction, large doses of iodids by ingestion and small, frequently repeated doses of neoarsphenamin by intravenous injection are generally indicated. If inunctions cannot be given, intramuscular injections of the salicylate or succinimide of mercury or bismuth may be substituted; if iodids are not well tolerated by ingestion, they may be tried by rectal injection or better still, sodium iodid may be given by intravenous infusion.

Specific Treatment of Syphilis of the Kidneys.—In view of the vascularity of the kidneys and especially of the rich capillary network of the glomeruli, their functional activity and great susceptibility to various tissue injuries, all of which would appear to favor the localization of *Sp. pallida*, it is a matter for wonderment that these organs are not involved more frequently than is indicated by the incidence of clinical detection during life and by macroscopic examinations after death. *Acute syphilitic nephritis* is occasionally recognized, but chronic or late syphilitic nephritis in the form of vascular lesions, diffuse interstitial fibrosis or localized fibrosis (gumma) is distinctly rare, insofar at least as clinical detection is concerned, although it is possible and, indeed, quite probable, that not a few cases of albuminuria ascribed to antisyphilitic medicaments are in reality due to syphilitic lesions of the kidneys. The occurrence of albuminuria and other evidences of renal irritation or inflammation may have the same varied etiology and significance in relation to specific treatment as jaundice in hepatitis; for example, the urinary changes may be due (*a*) to syphilitic infection of the kidneys and especially if the albuminuria is extremely high and occurring in the first two to six months of syphilis without treatment; (*b*) to chronic syphilis of the kidney; (*c*) to antisyphilitic drugs and especially mercury, and (*d*) to nephritis of non-syphilitic origin. Furthermore, insofar as albuminuria itself is concerned, it is not to be overlooked that this may be due to syphilitic cystitis and not to the nephritis at all.

In the *diagnosis* of acute syphilitic nephritis, Fournier[40] stated that there should be (1) proof of a recent syphilitic infection; (2) the appearance of nephritis with syphilis; (3) a lack of other sources for the nephritis, and (4) such marked evidences of nephritis as very high albuminuria, rapid onset and a tendency to early uremia. To these we may now add a positive blood Wassermann reaction and possibly the presence in the urine of Munk's[41] double refractile lipoids, which, according to Stengel and Austin,[42] may have a syphilitic significance. Unfortunately, Wassermann tests with the urine are not especially worth while and an examination of the urine for spirochetes is of limited value, since these may be present as a result of an exanthem of the bladder wall. Excellent clinical descriptions of this type of nephritis have been published by Munk[43] MacDonald,[44] Hoffmann,[45] Audrey[46] Stokes[47] and Cole;[48] insiduous and fairly rapid onset during the first six months after the chancre and usually about the fifth month, rapid loss of weight, anemia, edema, high albuminuria and Munk's lipoids (as detected by the polarizing microscope) are the chief changes.

In the *treatment of* acute syphilitic nephritis the diet should be bland and sufficiently liberal for raising or at least maintaining general nutrition with a restriction of the proteins. Specific medication is indicated and this should be as vigorous as possible under the circumstances, since a high mortality has been recorded. Most physicians have employed mercury by inunction or injection, but both Stokes and Cole have reported the recovery of cases treated with arsphenamin. It appears to me that the latter is the more rational therapy and since arsphenamin is somewhat less renotoxic than neoarsphenamin, it would appear to be the drug of choice, although the difference in this respect is not so great but that neoarsphenamin may be safely employed. Probably epifascial or intramuscular injections of sulpharsphenamin may be still better since absorption is slower with more gradual elimination. Stokes is of the opinion that arsphenamin is no more dangerous than mercury; since the latter is certainly more toxic for the kidney epithelium, I should say that it is distinctly less dangerous in small doses. The effect of

arsphenamin is likewise much more prompt. The iodids are not required. The treatment of this type of nephritis is, therefore, much like that outlined on page 957 for secondary syphilis, modified, however, to meet the condition of the kidneys by reducing the dosage of the arsenicals: arsphenamin 0.1 to 0.2 gm. and increasing to 0.4 gm. at weekly intervals; neoarsphenamin 0.3 to 0.4 gm. and increasing to 0.6 gm.; sulpharsphenamin 0.2 to 0.4 gm. Courses of any of these should be followed by mercury after the disappearance of the albumin and the whole treatment should be sufficiently prolonged to attempt a radical extermination of the disease. It is scarcely necessary to add that the urine should be examined two or three times a week with quantitative albumin determinations; it is also well to estimate kidney function by occasional examinations of the blood for urea and total creatinin as a better means for adopting in time the special treatment demanded by the threatened onset of uremia. The phenolsulphonphthalein functional test may be likewise of service in this connection.

In the treatment of *chronic syphilitic nephritis* I believe that arsphenamin and its substitutes are likewise indicated, although the treatment may be along the general lines advised for chronic syphilis in general with such modifications in dosage and number of injections as may be indicated by individual conditions. Frequent urine examinations for albumin and casts and occasional blood examinations for urea, non-protein nitrogen and total creatinin are especially in order as guides for the kind and degree of specific therapy. Probably preference should be given to sulpharsphenamin by epifascial injection, because of its slower absorption, and to bismuth, because it is less renotoxic than mercury.

The treatment of syphilis complicated by non-syphilitic nephritis is considered on page 1050.

Specific Treatment of Syphilis of the Bladder and Organs of Reproduction.—To the best of my knowledge the specific treatment of early and late syphilis of the bladder and the organs of reproduction of both sexes is exactly as described for the treatment of early and chronic syphilis in general, since involvement of these organs does not raise any particular questions as to choice of drug and manner of administration, as in the case of syphilis of the liver, kidneys, cardiovascular and nervous systems. In gummatous syphilis the iodids are especially indicated and Thompson[49] states that in gumma of the prostate gland he has observed very satisfactory results from intravenous injections of sodium iodid. Local treatment of lesions of these organs is of little or no use.

Specific Treatment of Syphilis of the Bones, Joints and Lymphatic System.—The specific treatment of syphilitic infection of the bones, joints and lymphatic system is that of early and late syphilis in general, since infection of these organs does not involve any particular points of importance. Indeed, syphilis of the bones is regarded as giving a particularly good prognosis, although syphilis of the joints is much more difficult to cure. According to Horwitz,[50] primary syphilis of the joints in acquired infections is rare and is usually an extension from the shafts; Lacapere and Laurent,[51] however, have observed joint involvements in about 10 per cent. of cases of syphilis in the natives of Morocco, and since the circulation of the synovial membranes and the chances of trauma are such as to greatly favor bacterial embolism, it is a wonder that the joints are not more frequently involved than generally observed. Poehlmann[52] states that in acquired syphilis, about 3 to 5 per cent. of cases have joint complications in the late stage, but these figures are probably a little too high; in children especially the disease may be mistaken for tuberculosis and lead to mutilating operations. The Wassermann reac-

tion is of great value in aiding a diagnosis; tests with joint fluid are not positive unless the blood reaction is positive.

Syphilis of the bones, occurring both as a periostitis or osteomyelitis, responds quite promptly although dead bone must be removed, absorbed or discharged before complete healing occurs. Charcot joints are especially refractory and at best one can but hope for a cessation of progress of the synovitis and arthritis. Periosteal swellings usually disappear, but may leave a residual thickening; lymphatic glands likewise subside, although a fibrotic thickening frequently remains for many years after being apparently sterilized of *Sp. pallida*.

As a general rule, the prognosis in periosteal and bone syphilis is very good, but chronic arthritis is far more refractory. In treatment, the organic arsenicals, mercury (especially by inunction) and large doses of the iodids are indicated after the method given in Chapter LXXIII for the treatment of chronic syphilis.

Specific Treatment of Syphilis of the Skin and Mucous Membranes.—As a general rule, early and late syphilitic lesions of the skin and mucous membranes respond rapidly to specific medication; this is especially true of the early lesions constituting the so-called secondary stage of syphilis. The treatment advocated is that described on page 957; since the lesions are infectious and those about the genital organs and in the mouth especially dangerous from this standpoint, the indications are for rapid sterilization by means of the administration of arsphenamin or its substitutes.

Chronic lesions of the skin, and especially of mucous membranes in the mouth and about the genitalia and anus, are frequently quite refractory to specific medication. Mucous patches and condylomata sometimes resist surprisingly large amounts of antiluetic drugs, but in this connection it is to be remembered that condylomata are not always of spirochetic origin and that patches in the mouth may be due to aphthous stomatitis as well as to spirochetic infection. In indolent ulcerative skin lesions, I have sometimes observed that a few intramuscular injections of 5 c.c. of sterile milk or one of the commercial milk preparations, greatly aided the effects of specific drugs. In chronic lesions the iodids are especially useful and the treatment may be that described for chronic syphilis in general. If eczema or other non-syphilitic skin lesions are present, due care must be taken in the administration of arsphenamin and its substitutes to reduce the chances of producing arsenical dermatitis.

Specific Treatment of Syphilis of the Glands of Internal Secretion.—In syphilis of the suprarenal, thyroid and other glands of internal secretion not already considered, there are no special precautions in specific therapy except possibly in the case of involvement of the thyroid gland with symptoms of hyperthyroidism, when the iodids should not be administered at all or but in small and carefully guarded doses to prevent sudden increase in the thyroid secretions. As mentioned in Chapter LVII, unsuspected adenomas and other involvements of this gland have been brought to the surface clinically, as it were, by the signs and symptoms of hyperthyroidism following the administration of the iodids in the general treatment of syphilis. Possibly we do not keep this in mind as much as we should and especially in the treatment of syphilis of women.

Syphilis of the suprarenal glands is not at all uncommon, as shown by the pathological studies of Warthin; as a general rule, the glands are especially heavily infected in congenital syphilis, myriads of spirochetes being found in sections. Some writers have recently stated that the asthenic states of acquired syphilis, and especially during the secondary stage as well as certain

pigmentations of the skin, are due to spirochetic infection of these glands. The specific treatment, however, is that of early or late syphilis in general, although, if arsphenamin or its substitutes are employed, it is well to be on guard for the occurrence of "nitritoid" reactions (Chapter LI) which Milian and others believe may be due to a sudden deficiency of adrenalin in the blood.

Specific Treatment of Syphilis of the Respiratory Organs.—As is well known, syphilis may involve any portion of the respiratory tract from the nose to the pleural surfaces, including the mediastinal glands. Chancres occasionally occur in the nose; I have seen two cases within the last three years, one in a physician. Chancres have been described in the larynx, but these are extremely rare and of course do not occur at all in the lower respiratory tract. Chronic syphilis of the nose and especially of the nasal bones is one of the best known clinical types; chronic syphilis of the larynx with or without ulceration is likewise well known. Syphilis of the lungs, however, is not recognized clinically in anything near like the frequency of its occurrence; doubtless many cases are regarded as early or closed cases of tuberculosis.

The specific treatment of early and late syphilis of the respiratory organs does not offer any special difficulties or problems; in syphilitic laryngitis, however, due care must be exercised in preventing Herxheimer reactions by initiating treatment mildly with mercury or bismuth and the iodids. I have seen several serious reactions follow the intravenous administration of 0.6 gm. doses of arsphenamin at the outset of treatment. The huskiness of chronic syphilitic laryngitis can be improved by specific medication, but in my experience, is seldom entirely corrected, if present for several years; progressive changes can be prevented, but the fibrous thickening or paralysis of the cords generally persists.

I have known of several cases of tuberculous laryngeal ulcers mistaken for syphilis; great care must be exercised against falling into the error of assuming that all such lesions are syphilitic because of positive blood Wassermann reactions. If an ulcer does not show considerable improvement after a course of mercury, iodids and a few injections of arsphenamin or one of its substitutes, it is not likely to be a syphilitic ulcer.

The presence of tuberculosis of the lungs may complicate the treatment of syphilis, but this is a large and important subject considered with more detail on page 1051.

Specific Treatment of Syphilis of the Ear and Auditory Nerves.—As is well known, syphilis may involve any portion of the auditory system and of special interest, from the standpoint of both diagnosis and specific therapy, is involvement of the labyrinth and the eight or auditory nerve. The high incidence of otologic infection in syphilis is not generally appreciated, although the eighth nerve is now known to be involved much more frequently than formerly surmised as a result of the new labyrinth tests applied by Jones, Fischer and others. Indeed, Jones is of the opinion that eighth nerve mystagmus tests afford the earliest clinical evidence of syphilitic infection of the central nervous system and may indicate this involvement as early as the results of spinal fluid examinations and long before there are clinical evidences of involvement of the optic, oculomotor and other cranial nerves, when periodic tests show a mystagmus (after turning) of eighteen seconds (instead of the normal twenty-six seconds) followed later by one of twelve seconds, etc. Furthermore, these tests are believed to possess value as a guide in treatment and prognosis in that they may show either progressive impairment or improvement as the case may be. Owing to the fact that the labyrinthine artery is a direct branch of the basilar and equally susceptible to infection

with *Sp. pallida*, and since the eighth nerve in its wide ramifications is especially subject to involvement in syphilitic basilar meningitis, it is no wonder that deafness and other manifestations of otologic involvement are not uncommon in syphilis.

The specific treatment of syphilis of the external auditory apparatus and tubal stenosis from pharyngeal lesions, present nothing in the way of special difficulties. But when the auditory nerve is involved in basilar meningitis and especially with extensions to the bony foramina, the matter of Herxheimer reactions and so-called neurorecurrences or recidives, as a result of specific medication and especially with arsphenamin and its substitutes, becomes one of considerable importance.

I am quite sure that arsphenamin, neoarsphenamin and sulpharsphenamin have no direct toxic effects upon the normal or non-syphilized auditory nerve; neither have any reports been yet made of a toxic effect upon this nerve by tryparsamid or other pentavalent compounds, although I am prepared for such since recent reports on the use of tryparsamid in the treatment of neurosyphilis have shown objective and subjective impairment of vision in 5 to 10 per cent. of cases receiving six or more intravenous injections of 2 to 3 grams each. Although there are reports of ears with non-syphilitic labyrinthine involvement apparently made worse, and of syphilitic labyrinthine infections suddenly made stone deaf by the administration of arsphenamin and neoarsphenamin, yet I believe that these accidents were due to Herxheimer reactions of the eighth nerve as a result of an antecedent syphilitic involvement of this nerve, clinically undetected in the first group and known to be present in the second. These Herxheimer reactions have been described in Chapter LVIII; very little increase of exudate in the sheath of the nerve in its bony foramina apparently suffices for producing marked functional disturbances.

As recently stated by Babbitt,[53] great care should be exercised in the administration of arsphenamin and its substitutes when labyrinthine tests show involvement of the eighth nerve; but I believe this is demanded because of the dangers of producing Herxheimer reactions and not because the arsenicals produce a direct toxic effect upon the nerve analogous to the direct effects of atoxyl and tryparsamid upon the optic nerves. When nerve changes occur in early syphilis, as in the course of early infections of the meninges in the first six to twelve months of the disease, ordinary specific treatment, as described in Chapter LXXII for early syphilis, is apparently well borne. But when specific treatment is undertaken two or more years after the chancre, it should be begun with mercury or bismuth and the iodids followed by cautious doses of arsphenamin, neoarsphenamin or sulpharsphenamin for the general treatment of chronic syphilis; this greatly reduces the chances of producing exacerbation of the syphilitic infection with an increase of exudation into the sheath of the nerve and consequent functional impairment. Since tryparsamid and other pentavalent arsenicals are certainly more neurotoxic than arsphenamin and neoarsphenamin, they should not be employed in treatment until more information is available regarding their effects upon the auditory nerve, unless the case is one of paresis, taboparesis or other type of grave neurosyphilis demanding tryparsamid therapy even in the presence of possible injury to the optic or auditory nerves. Indeed, the whole subject of the treatment of syphilis of the internal ear is very closely related to that of the treatment of neurosyphilis in general, which is discussed in the following chapter.

Specific Treatment of Syphilis of the Eye and Optic Nerves.—The specific treatment of early and late syphilis of the eye and optic nerves is essentially

similar to that described for the treatment of syphilis in general. While mercury and the iodids were formerly the chief drugs, there is now a growing consensus of opinion that arsphenamin and neoarsphenamin are to be preferred in practically all cases in association with mercury and iodid. While primary syphilis of the eyelids, conjunctival and cornea is rare, secondary and tertiary lesions are quite common and may involve any of the deeper structures, with the exception of the lens, and particularly the iris, cornea and uveal tract. Involvement of the optic nerves is likewise common and especially in the form of primary atrophy associated with tabes dorsalis; indeed, syphilis of the eye and of the optic nerves is one of the most frequent and important of visceral infections, and not infrequently the presence of the disease is first detected or suspected in the course of ophthalmic examinations.

Examinations of the eye and especially of the fundus should be almost a routine measure before the institution of specific therapy in late syphilis and especially if the organic arsenicals are to be administered. With arsphenamin, neoarsphenamin and sulpharsphenamin these examinations are advisable for the purpose of detecting involvement of the optic nerves in order that their administration may be so regulated to prevent Herxheimer reactions; as will be shortly discussed, there are no reasons at all for suspecting that they exert a direct toxic influence upon the nerves. However, when the pentavalent organic arsenicals like tryparsamid are to be administered, there is an important additional reason for a very careful opthalmic examination because these drugs are capable of injuring the nerves.

In other words, when arsphenamin was first introduced by Ehrlich for the treatment of syphilis, it was received by the ophthalmologists with considerable fear and hesitation since the toxic amblyopias of atoxyl, arsacetin and arsenophenylglycin were still fresh in mind. And true to expectations visual disturbances were soon reported, but it was quickly found that the eye changes were different from those produced by atoxyl. The fundi commonly showed inflammatory exudates in the retina and choroid, associated with hyperemia of the vessels, vitreous opacities and sometimes with hemorrhages; in short, the lesions were due to reactivated syphilitic deposits instead of an essentially degenerative process affecting either the ganglion cells of the retina or the fibers of the optic nerve as produced by atoxyl. Furthermore, but one eye is usually involved, whereas both eyes are usually involved in atoxyl amblyopia and above all, the further administration of arsphenamin is found to clear up the lesions in the majority of cases whereas the additional administration of atoxyl intensified its lesions. Furthermore, while atoxyl amblyopia develops early, the Herxheimer and neurorecurrences of arsphenamin usually develop one or two months later because time is required for the laying down of the increased syphilitic exudates responsible for the lesions and disturbances. In short, the usual disturbances produced by arsphenamin and neoarsphenamin are due to an exacerbation of latent syphilitic involvements of the optic nerve; the visual disturbances of atoxyl are sometimes due to the same process plus, in addition, to a degeneration toxic effect upon the nerve fibers. However, this is now an old story, since the differentiation was made so clearly by McAdams[54] and others ten years ago, but I note in some text-books on ophthalmology that atoxyl is still mentioned for the treatment of syphilitic lesions of the eye. This is a serious mistake because the curative effects of atoxyl are so much less than arsphenamin, neoarsphenamin and sulpharsphenamin that it has no place in the modern treatment of syphilis; furthermore, it has the great disadvantage of producing optic degeneration. It is true that Pomaret and others especially interested in developing the pentavalent arsenicals for the treatment

of syphilis, claim that these effects are due to other impurities in atoxyl. I have already discussed this subject in Chapter XLVI, and believe their claim is erroneous in view of recent experiences with tryparsamid in addition to the results of an investigation by Loevenhart and Young,[55] who showed that arsenicals with an amino-group, or substituted group in a para-position to the arsenic, produced lesions of the optic nerves in rabbits, whereas organic arsenicals with the animo-group in the ortho- or meta-position to the arsenic produced no lesions of the optic nerves in these animals. It would appear therefore, that pentavalent compounds in general are more apt to attack the optic nerve than the trivalent arsenicals. Interest in this subject has been aroused again with the past two years by the use of tryparsamid for the treatment of neurosyphilis and while Lillie believes that the visual disturbances ascribed to it are in reality syphilitic lesions, yet Lorenz and this associates, Woods and Moore, and others, are apparently convinced that tryparsamid may injure the optic nerves and that it should not be administered at all, or very cautiously, when visual disturbances are present. I predict, however, for this drug, a useful place in the treatment of paresis, tabes and taboparesis, and ophthalmologists may expect to be frequently called upon for an opinion of the condition of the optic nerves before it is administered; this subject will be, however, discussed at more length in the succeeding chapter.

In the treatment of syphilis of the eye and optic nerves, arsphenamin, neoarsphenamin and sulpharsphenamin are the remedies of choice; atoxyl and tryparsamid should not be used if the optic nerves show involvement. But it may be asked, are not the Herxheimer reactions excited by arsphenamin and its substitutes equally dangerous? Experience has shown that they are not and that they may be cleared away with addititional specific treatment. Furthermore, they are not to be feared at all in early syphilis of the eye, as in chancres of the external parts or the iritis of the secondary stage. But in all cases of more than a year since infection, the possibility of a Herxheimer reaction is to be kept in mind and especially when previous treatment has been insufficient or none at all has been given. Can they be avoided? In the majority of cases this appears possible by initiating treatment with mercury or bismuth and iodids for a month or two before the administration of the arsenicals is begun, which I believe is the correct principle of treatment of practically all cases of late syphilis as described in Chapter LXXIII. But why not use mercury and the iodids alone in the treatment of syphilis of the eye and optic nerves and thereby make sure of avoiding Herxheimer reactions and optic nerve degenerations? In the first place mercury may produce the same reactions as arsphenamin but less frequently. In the second place, the majority of ophthalmologist appear convinced that mercury and iodids alone are insufficient and far inferior to arsphenamin and its substitutes. Therefore, since the latter compounds do not produce toxic amblyopias and are the superior spirocheticides, and since Herxheimer reactions can be avoided in the majority of cases by proper administration, they are the remedies of choice in the treatment of ocular syphilis, not alone, however, but in judicious combination with mercury, bismuth and the iodids. As stated by Young[65] mercury alone frequently fails to either check or cure the ocular lesions of syphilis and that arsphenamin and neoarsphenamin are the remedies of choice. As is usual in the treatment of syphilis in general, the latter drugs are administered by intravenous injection and sulpharsphenamin by intramuscular injection. Lamb[57] has advocated the treatment of syphilitic iritis, iridocyclitis, kerato-iritis and interstitial keratitis by subconjunctival injections of 1 c.c. of Swift-Ellis serum, but I am convinced

that the beneficial effects are simply due to non-specific protein reactions, since the amount of arsphenamin contained in this amount of serum is certainly too small for spirocheticidal effects. Treatment of syphilitic optic neuritis and especially of primary atrophy, is also conducted by Swift-Ellis intraspinal injections, but I shall refer later to this subject in more detail.

With these introductory remarks, I may now briefly consider the specific treatment of some particular syphilitic lesions of the eye, although it is to be carefully borne in mind that the treatment should be for syphilis in general since the eye lesions are never unaccompanied by lesions elsewhere and particularly of the cardiovascular and central nervous systems. Indeed, the ophthalmologist who treats syphilitic lesions of the eye alone to the disregard of the rest of the body is more reprehensible than the physician who treats chronic syphilis of other organs while disregarding the eye. As a matter of fact the specific treatment of primary optic atrophy is principally concerned with the treatment of tabes dorsalis and other types of syphilitic meningitis and not of the eye itself at all; for this reason the subject of specific treatment is fully considered in the following chapter, but I shall also discuss it here in a brief manner because of its particular interest to the ophthalmologist.

In the specific treatment of *acute early syphilis* of the eye, like chancres of the lids, conjunctivæ and cornea as well as the iritis of the secondary stage, exactly the same measures may be taken as described in Chapter LXXII for the treatment of early syphilis in general. The indications are to check the lesions as quickly as possible by intensive medication. To temporize with mercury and the iodids is a mistake unless some contra-indication to the arsenicals is present of which the only one I can think of in this connection is allergic hypersensitiveness to arsenic. There need be no fear of Herxheimer reactions or degeneration of the optic nerve in these early cases if arsphenamin, neoarsphenamin or sulpharsphenamin are employed. Additional measures are of course required; in chancres, for example, cleansing and local treatment are indicated. In acute iritis, hot fomentations or dry heat and leeches may be indicated for the relief of pain and especially the instillation of atropin for mydriasis until all ciliary irritation has subsided and during the period of changes in the refractive power of the eye. These measures, however, should be always under the control of the ophthalmologist.

In *parenchymatous* or *interstitial keratitis* as well as in *keratitis punctata syphilitica*, best results are observed from mixed arsenical, mercurial and iodid therapy. The older forms of treatment with mercury and iodids alone have more frequently failed than succeeded. For the 2 to 10 per cent. of cases due to acquired syphilis, specific medication may be along the lines described for the treatment of chronic syphilis. For the 80 to 90 per cent. of cases due to congenital syphilis, the treatment described in Chapter LXXVII may be employed, which is essentially similar except that the doses and administration are modified to suit children. Subconjunctival injections of mercury have been used, but I am sure that these are beneficial only insofar as they produce congestion and other non-specific reactions similar to injections of salt solution and sodium saccharinate (Fox). My experience with systemic medication has been so satisfactory that I very much doubt the wisdom and practical value of subconjunctival injections; when non-specific protein stimulation has been indicated, intramuscular injections of sterile milk or one of the commercial milk preparations have yielded the desired effects in my experience. Local treatment in the form of instillations of atropin or dionin may be required to maintain mydriasis and allay inflammation and pain, unless a rise of tension appears when mydriatics should be discontinued.

The specific treatment of other chronic syphilitic lesions of the eye may be along the same lines as advised for chronic syphilis in Chapter LXXIII. It is particularly important to start treatment either with mercury or bismuth and the iodids or cautiously with the arsenicals in order to minimize the chances of producing Herxheimer reactions of the optic nerve. This is especially true if ophthalmoscopic examination discloses evidences of optic neuritis which, according to Igersheimer,[58] may be differentiated from choked disc of intracranial pressure by the presence in the former of vitreous opacities, central scotomas and decrease in dark adaptation.

Of special interest is the specific treatment of *primary optic atrophy*, which is so often associated with tabes dorsalis as to be known as *tabetic atrophy*. As stated above and especially shown by Fordyce and Rosen,[59] there is no proof at all that arsphenamin acts adversely upon the optic nerve; on the contrary its administration frequently arrests the progress of this dreadful lesion and Würdemann[60] has had a similar experience. Fordyce[61] has had particularly good results in the treatment of this condition by a combination of intravenous and intraspinal medication according to the Swift-Ellis method, and states that, in 33 cases, 9 of which were simple optic atrophies, 17 tabetic and 7 paretic types, this treatment resulted in the arrest of the disease and improvement in 12 cases (5 for 2 to 5 years) in a series of 17 with incomplete atrophy of both eyes; in 7 cases with complete atrophy of one eye and partially of the second, 2 progressed and 5 were arrested, while in 8 cases with complete atrophy of both eyes before treatment there was, of course, no improvement. In other words, specific medication cannot do the impossible in the way of restoration of completely destroyed nerves, as the laity and not a few of the profession appear to expect; but if the progress of the syphilitic infection is stopped, nerve fibers partially crippled may recover with the removal of spirochetes and absorption of exudates, with consequent improvement in vision or at least a cessation of further impairment. But this subject intimately concerns the specific treatment and prognosis of neurosphilis in general and I shall continue with its discussion in the following chapter.

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CHAPTER LXXVI

SPECIFIC TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM (NEUROSYPHILIS)

Probably the most important type of visceral syphilis is infection of the central nervous system, not only because it may equal in frequency syphilitic infection of the cardiovascular system and other organs, but because of the seriousness of the chronic infections of the meninges, brain, cord and such of the cranial nerves as may be involved in basilar meningitis. Indeed, cardiovascular and neurosyphilis are the two most common and important types of visceral syphilis and the treatment of the latter is sometimes modified to meet the demands of the former, as when preference is given the Swift-Ellis or some other method of intraspinal treatment when neurosyphilis is complicated by such severe myocardial infection as may render dangerous the intravenous injection of large amounts of arsphenamin or neoarsphenamin. Lowenberg[1] states that in 30 to 40 per cent. of cases of neurosyphilis, some type of syphilitic involvement of the aorta will be found and Stokes, Moore and others have expressed similar opinions.

What is Neurosyphilis?—In a strict sense "neurosyphilis" means the infection of any nervous tissue with *Sp. pallida*. As stated in Chapter XLII, however, I believe that a distinction may be made between *invasion* of these tissues with *Sp. pallida* and *infection* of them. In the former, the parasites are brought to the tissues by the blood and lymphatic circulations, but they do not lodge permanently even though their temporary residence may produce slight but temporary spinal fluid changes and constitute what Nast[2] has so aptly designated as "spinal fluid syphilis" when applied to the spinal fluid changes in early syphilis. In late syphilis, however, the spinal fluid may likewise show profound changes without clinical symptoms constituting "asymptomatic neurosyphilis," sometimes what has been called "asymptomatic paresis," and surely indicative of profound infection. But in early syphilis I believe that temporary residence of *Sp. pallida* may be terminated by natural immunological forces, since it is reasonable to surmise that the parasites are brought to these tissues during the spirochetemic stages of syphilis, while marked spinal fluid or clinical evidences of infection are found in not more than 40 to 60 per cent. How and why the others escape are questions that involve the matter of specific tissue strains of *Sp. pallida*, hereditary and acquired reduction of tissue resistance, etc., which have been already discussed in preceeding chapters.

Since syphilitic infection of the peripheral nerves is rather uncommon, the term "neurosyphilis" has become, by reason of common usage, practically confined to infection of the brain, cord, meninges and such of the cranial nerves as may be involved inside of the cranial cavity by these processes and especially in basilar meningitis, or in their bony canals. The term has replaced the older words "metasyphilis" and "parasyphilis" of the brain and cord, since it has been established that the diseases are due to the actual presence of the parasites rather than to various other agencies held responsible prior to the discovery of *Sp. pallida* itself by Schaudiun and especially the presence of the parasites in the brain and cord by Noguchi and Moore. Neurosyphilis, therefore, as commonly applied, refers to infection of the meninges, brain, cord and cranial nerves with *Sp. pallida* in which the evidences are expressed by spinal fluid changes, by signs and symptoms or, most commonly, by a combination of these.

Incidence of Neurosyphilis.—During the past ten years numerous investigators have shown that spinal fluid changes of varying degree occur in as high as 60 per cent. of *untreated* cases of syphilis during the first six to twelve months of the disease; as a result of adequate specific medication during this first year, the incidence is ordinarily reduced to about 25 per cent., this subject having been reviewed in detail in Chapter XLII. Just what proportion of syphilitics treated by the older methods, consisting of the administration of mercury and iodids after the methods of Fournier, Hutchinson and others, show laboratory and clinical evidences of neurosyphilis, cannot be stated, but it is probable that the incidence is in the neighborhood of 40 per cent. under such methods of specific medication. At the present time, about 20 to 40 per cent. of uncured cases of chronic latent syphilis will show spinal fluid abnormalities, the lower figure (20 per cent.) occurring among those known to have had intensive treatment with arsphenamin and mercury during the first year of the disease. Our figures on the incidence of neurosyphilis, based entirely upon clinical signs and symptoms, are much less definite because the detection of these varies so greatly with the knowledge and experience of the physician making the examination. If one were to base an estimate upon the incidence of such well-defined types as tabes, paresis, tabo-paresis, cerebrospinal syphilitic meningitis (diffuse and localized

including the cranial nerve involvements) and such vascular lesions as the thromboses and hemorrhages, the incidence would be probably in the neighborhood of 10 per cent., as recently estimated by Solomon.[3] But when one recollects in this connection the minor signs and symptoms as well as the atypical cases, that may be detected by expert neurological syphilologists, I am inclined to believe that what may be called the *clinical* incidence of neurosyphilis, is in the neighborhood of 10 to 20 per cent. of cases of chronic syphilis and therefore not very much below the incidence of *spinal fluid* syphilis.

Is the Incidence of Neurosyphilis Increasing? *Neural Infection in Relation to Specific Therapy.*—This raises the question whether the incidence of neurosyphilis is increasing. Undoubtedly the *detection* of neurosyphilis during the past ten years has greatly increased owing to advancing knowledge of the clinical manifestations and spinal fluid changes. But is the incidence actually increasing over what it was fifteen years ago and before the introduction of arsphenamin and its substitutes for the treatment of syphilis? Many physicians have stated that this is actually the case and have directed attention to the increasing frequency of paresis or preparetic states in comparatively young men and women within a few years after infection. Others like Roch[4] believe that arsphenamin and its substitutes stimulate latent foci of infection into activity; others believe that rapid destruction of spirochetes in the early stages by the organic arsenicals prevents antibody production or other immunological changes favoring resistance and latency with the result that spirochetes escaping destruction are better able to infect the central nervous system; still others believe that the vascular effects of arsphenamin produce capillary injuries in the brain, cord and meninges favoring the dissemination and lodgement of the spirochetes. Hata[5] states that the incidence has certainly not been decreased by treatment with arsphenamin and Fordyce and Rosen[6] believe that there has not been a true increase during the past ten years, but only an apparent increase due to more systematic investigations and a more thorough knowledge of this special type of syphilis.

I believe that it is highly probable that the incidence of clinical and spinal fluid neurosyphilis has actually increased during the past fifteen years, largely if not entirely as a result of wrong methods for treating both early and late syphilis with arsphenamin and its substitutes. For the first five to ten years after the introduction of arsphenamin, the drug was not usually given in sufficient amounts; it was altogether a too common practice to depend upon one to four injections which usually sufficed for dissipating the manifest lesions and even reversing temporarily insufficiently sensitive complement-fixation reactions from positive to negative. But apparently one result has been that, in not a few cases of early syphilis so treated, the consequent reduction in the number of spirochetes depressed immunological reactions and resistance enabling the surviving parasites to produce more widespread infection including the tissues of the central nervous system. From my histological studies I have seen no evidences of vascular injuries from arsphenamin and neoarsphenamin in the doses ordinarily employed in our intensive methods for the treatment of early syphilis; neither are there any evidences of adverse effects upon such of the cranial nerves so commonly involved in neurosyphilis, as the optic, oculo-motor and auditory nerves. For these reasons I believe that the adequate treatment of early syphilis, by not a few, but a large series of injections of arsphenamin and its substitutes, is the best guarantee against the subsequent development of both spinal fluid and clinical neurosyphilis, as discussed in Chapter LXXII, and maintained

by the majority of syphilologists including Fordyce and Rosen, Swift, Stokes, Schamberg, Sicard, Gennerich, Wechselmann, and others mentioned in preceeding chapters, as well as Harrison,[7] Mott,[8] Riddoch,[9] Scott and Pearson,[10] Christian,[11] Lowrey[12] and numerous other writers in America, England, Germany, France, Italy and other parts of the world. As between Fournier's old chronic, intermittent but thorough and prolonged mercurial medication and present-day haphazard, too brief and inadequate treatment with a few injections of arsphenamin or neoarsphenamin, I believe the former is the better treatment in the final analysis and especially from the standpoint of infection of the central nervous system; but with more intensive and adequate methods for the treatment of early syphilis with arsphenamin and its substitutes, I believe that Fordyce is correct in his belief and statement that the incidence of neurosyphilis has not actually increased.

In chronic syphilis, however, arsphenamin and its substitutes are so much more likely than mercury to stimulate latent foci of infection into activity, that is, produce neural Herxheimer reactions, but there can be no denial of the fact that these compounds have increased the incidence of such neurorecurrences when administered to cases of chronic latent syphilis in large doses by intravenous injection; even latent cases of tabes dorsalis and other infections of the cord and brain may be stimulated into activity by intravenous injections of large doses of arsphenamin. These in turn may conceivably favor the spread of the infection from these foci and especially if the follow-up therapy is inadequate for checking the renewed spirochetic activities. These effects, however, are not peculiar of arsphenamin and neoarsphenamin alone; mercury and other spirocheticides have a similar action when given in large doses. But the fact remains that arsphenamin and its substitutes are most likely to produce neural Herxheimer reactions or neurorecurrences and neurorecidives with the result that their incidence has increased during the past ten years. But these untoward effects are usually preventable by initiating the treatment of chronic syphilis with mercury or bismuth, very small doses of arsphenamin or with intramuscular injections of sulpharsphenamin followed by larger doses. In the treatment of both acute and chronic syphilis, therefore, the manner or method of administering arsphenamin or its substitutes apparently bears a relation to the incidence of neural infections.

Some writers have maintained that the old custom of allowing the secondaries to develop for diagnostic purposes before mercurial treatment was instituted, resulted in fewer neural and cardiovascular infections. This may be actually true since such practice afforded maximum opportunities for the development of immunological defenses, but the incidence of neural and cardiovascular syphilis under this plan was so high that no one should seriously consider a renewal of the practice in preference for our present day methods for the intensive treatment of early syphilis by which there are excellent chances for the complete extirpation of the disease. As stated in Chapter LXXII, I believe this is the sensible plan when the general condition of the patient admits intensive medication, as is true with the great majority of early syphilitic infections. But a few haphazard injections of neoarsphenamin may be actually harmful in the final analysis because they kill off only enough spirochetes to dissipate the external lesions without complete sterilization, with the result that the patient is denied the benefit of maximum immunological defense with the increased danger of wider dissemination of surviving parasites; hence the great responsibility of physicians undertaking the treatment of early syphilis and the necessity for thorough and intensive specific medication.

Time of Occurrence of Neural Infection.—As discussed in Chapter XLII, numerous investigators have shown that spinal fluid changes indicate that the tissues of the central nervous system and especially the meninges, are invaded by *Sp. pallida* in the majority of cases of early syphilis unless the infection is intensively treated during the chancre stage. If the disease is untreated until the secondaries appear, I see no reason for doubting that in practically all cases the spirochetes are brought to these structures by the blood and lymphatic circulations although spinal fluid evidences may not be found in more than 70 per cent. because the invasion in some cases meets with sufficient resistance on the part of the tissues to prevent permanent lodgement or infection. The specific treatment of primary and secondary syphilis apparently results in the cure of many of these early neural infections to the extent that spinal fluid abnormalities are reduced to about 25 per cent. in the first two years of the disease and additional treatment frequently results in the further reduction of this incidence; certainly, as stated by Wiesel[13] and others, cases of precocious neurosyphilis, with the development of clinical signs and symptoms of neurosyphilis and abnormal spinal fluid changes within a few years after the chancre, are the most difficult cases to control and require a most intensive therapy in which it is well to combine intraspinal with intravenous medication, as advised by Gennerich.

Fordyce and Rosen[14] are convinced that, if the spinal fluid shows no abnormalities at the end of this time, the patient can be assured that neurosyphilis will not develop in the future and this greatly aids the mental comfort of many individuals who have prominently in mind the terrors of paresis and tabes dorsalis. Finger and Kyrle[15] and others mentioned in Chapter XLII also state that negative spinal fluid findings at the end of the secondary stage tells the story and that a reversal to positive during subsequent years is of rare occurrence. Indeed, most students of this subject have subscribed to these views and, since the late Dr. Fordyce[16] has had a particularly rich experience and has been able to follow up some cases for at least ten years, his opinion commands the utmost confidence and respect. It is always advisable, therefore, to examine the spinal fluid at the close of the treatment of early syphilis because negative findings are so important that the mental relief and comfort more than compensate for the inconvenience attending the spinal puncture, whereas if positive findings are obtained, it is important to continue specific medication.

But I doubt the wisdom of being too sure about the matter of subsequent neural involvements. If we could be absolutely sure of the complete eradication of all early infections, the matter would be closed of course in a final and definite manner, but such is not the case. Negative spinal fluid examinations are not an absolute criterion of absence of infection of the tissues of the central nervous system, just as the clearing up of abnormal changes is not an absolute criterion of cure of neural infection. Deep parenchymatous infections may not show spinal fluid changes; neither may a few, scattered, light and early superficial vascular and localized meningeal involvements. In other words, it may be possible for small latent foci of residual infection to escape detection; I know positively that two cases in my own practice showed spinal fluid changes two and three and a half years, respectively, after negative findings at the end of the first year of intensive treatment of early syphilis. Furthermore, I believe that periods of spirochetemia may occur during the chronic stages of syphilis, that is, the presence of spirochetes in the blood as indicated by the positive results following the injection of rabbits with the blood of such cases, and I cannot understand why the central nervous system may not become infected at this time, even though these tissues apparently escaped

during the early or initial period of spirochetemia. Unquestionably the experience of Fordyce and his colleagues justifies us in stating to those individuals showing no clinical evidences of spinal fluid abnormalities of neurosyphilis, that in all probability they have escaped this type of infection and assuring them with considerable confidence that they need not fear subsequent infection even though there may be residual infection somewhere in the body, but we must avoid being too dogmatic and sure since it may not be possible to detect small or latent foci in the central nervous system which may spring into activity at a later time as stated by Vincent;[17] neither should we neglect to carefully examine the central nervous system for evidences of such when patients return at intervals for examination. We should not neglect additional spinal fluid examinations when there are the slightest reasons for suspecting neural involvement with negative blood Wassermann reactions, even though the spinal fluid on a former occasion showed no abnormal changes.

Pathology of Neurosyphilis in Relation to Specific Therapy and Prognosis.—Nothing is more essential for a proper understanding of the problems and difficulties of the treatment of neurosyphilis, and for a correct understanding of its possibilities and prognosis, than a good working knowledge of the fundamental pathological changes. Numerous elaborate and valuable histopathological studies have been made with the introduction of many new terms describing the finer points of cellular changes which tend to confuse the subject, but comparatively few of the essential facts concerning the chief tissue changes suffice for the present purposes and the clinico-pathological classification given on page 524 has been found to fulfill practical needs in this subject.

It would appear that *Sp. pallida* may attack any portion of the central nervous system although some portions are infected much more frequently than others. For example, infection of the parenchyma of the cerebrum and overlying meninges is common, usually resulting in a symptom-complex designated as paresis; the nerve cells of the cerebellum, however, are much less frequently involved. In the spinal cord the parasites frequently attack the posterior nerve roots with subsequent ascending degeneration of the posterior columns, producing the symptom-complex known as tabes dorsalis, whereas the motor nerves and anterior horn cells escape, the whole disease being one of the sensory system. Not infrequently the histopathological changes detected after death are much severer than estimated during life, because infection may occur in areas of lesser physiological importance and producing either no signs or symptoms at all or signs which are no detected or classified at present owing to deficiencies in our knowledge of the functions of some portions of the brain. Regarding the essential pathological changes in the meninges, blood-vessels and parenchyma, I can do no better than refer the reader to an excellent recent resume of the subject by Solomon;[18] also brief reviews recently published by Kaliski and Strauss,[19] Redlich,[20] Grinker,[21] Stoll,[22] and others in relation to diagnosis and treatment.

From the standpoint of treatment, the most satisfactory results are observed in the meningeal and meningovascular types of the disease. These include the large number of syphilitics showing spinal fluid changes in early syphilis but without symptoms, who improve or apparently are cured during the course of routine treatment; also the types with symptoms commonly designated as cerebrospinal syphilis. In this category we may also include early cases of tabes dorsalis when the disease is largely in the form of localized meningitis of the posterior roots with various shooting pains in different parts of the body and before degeneration of the ganglia and posterior spinal

columns has occurred. With the subsidence of the meningeal changes under intensive systemic treatment, the irritative symptoms of excrutiating pains, bladder disturbances and various crises, frequently disappear quite promptly, with a return of the spinal fluid to normal. In other words, best results in the treatment of neurosyphilis are to be expected in the meningeal rather than the parenchymatous forms, apparently because the drugs are brought so intimately in contact with the parasites by the rich blood supply of the pia; also in acute rather than chronic meningeal and vascular types, before thickening of the capillaries has taken place. Even mild treatment with mercury and iodids may apparently suffice, although more intensive treatment with bismuth or arsphenamin or its substitutes, mercury and iodids assure a much larger percentage of recoveries. Some cases, however, are more refractory even to intensive intravenous therapy, although some of these respond to a combination of intravenous and intraspinal treatment.

The lesions of parenchymatous neurosyphilis of which paresis is the chief type, are much more refractory to treatment. Why this should be is not thoroughly understood. It is true that the brain is heavily infected as shown by histological and inoculation tests, but with such a powerful spirocheticide as arsphenamin, this numerically heavy infection should not be the important factor. Nor can it be ascribed to poor blood supply as the parenchyma of the brain is rather richly supplied. Since the vessels commonly display perivascular infiltrations with fibrosis, it may be that the escape of spirocheticides into the parenchyma is hindered. But the more important probabilities are that arsphenamin and its substitutes are not sufficiently lipoid soluble or diffusible in the brain and that mercury does not tend to accumulate to spirocheticidal concentrations as well as it does in connective tissue involvements. Occasionally, however, cases having all the clinical and laboratory signs and symptoms of paresis may respond in a surprising manner, leading to long remissions, with improvement of serological findings. As shown by Solomon and Taft,[23] antisyphilitic treatment of paretics may favorably affect the pathological changes. On the other hand, it would appear that histological changes, identical with those met with in paresis, may exist for years in parts of the brain not concerned with the intellectual functions, designated by the late Dr. Southard as "paresis sine paresi" and indicating the long latent or asymptomatic period which may exist in syphilis of the brain.

Experience has shown, therefore, that not a few cases of neurosyphilis and especially cases of the early meningeal or meningo-vascular types, respond favorably to intensive systemic treatment alone or to a combination of systemic and intraspinal treatment. Spirochetes are destroyed and exudates absorbed with consequent clinical and serological improvement. In not a few instances, however, cases of these kinds in whom we have reason to expect such results, fail to improve and every case must be regarded on its own merits.

It is very important to remember, however, that destroyed nerve cells cannot be replaced with restoration of function. Such is frequently expected in tabes dorsalis, optic atrophy, etc. If cells are simply incapacitated by the pressure of lymphocytes and plasma cells, the absorption of these may result in partial or complete restoration of function, so that some clinical improvement may follow specific treatment in paresis, tabes dorsalis and tabo-paresis. A common mistake, however, is to expect too much of specific therapy. In early asymptomatic involvement complete cures are apparently secured in a goodly percentage of cases, but in symptomatic cases of longer standing, the real and usually only hope is for checking the progress of the infection with relief from symptoms and the improvement of functional capacity for the usual span of life.

Diagnosis of Neurosyphilis in Relation to Specific Therapy.—It is not within the province of this treatise to consider in detail the clinical diagnosis of neurosyphilis; nor am I competent to discuss this broad, intricate and important subject. But I have had sufficient experience to be impressed with the necessity for every physician undertaking the treatment of syphilis to at least acquaint himself with the more important signs and symptoms of neurosyphilis and especially the early signs and symptoms, if he cannot have the aid of a colleague skilled in neurological diagnosis and especially what may be called a sub-speciality in this branch, namely, syphilitic neurology and psychiatry. Naturally, I have had more experience in serological diagnosis already discussed in Chapter XLII, but realize its limitations in both diagnosis and as a control on treatment, as discussed by Sachs,[24] Finlaysen,[25] Fordyce[26] and others.

Probably the most important phase of diagnosis in relation to treatment is the ability to detect the *early* signs and symptoms of neurosyphilis, since so much may be gained by appropriate treatment at this time. The recognition of well-developed paresis or tabes is not usually a difficult matter, but the diagnosis of early involvements is a matter requiring much experience and thorough clinical examination. In asymptomatic cases, examination of the spinal fluid is the only means for diagnosis; in cases presenting mild or indefinite signs and symptoms, these tests are also of great value. During the first two years of syphilis the symptoms may be quite varied as headache, vertigo, insomnia, nervousness, pains in the extremities, phobias, and inability to concentrate. Careful examination may show early optic and auditory involvement with irregular pupils and alterations in the patellar and other deep reflexes the subject having been recently covered in a masterly manner by the late Dr. Fordyce.[27]

Attempts to Increase the Penetration of the Tissues of the Central Nervous System by Spirocheticides.—Owing to the frequency of neural infections and the unsatisfactory status of the treatment of parenchymatous infections, it is not surprising that early and frequent attempts have been made to increase the degree of penetration of spirocheticides in the tissues and spinal fluid during systemic medication. Among the earliest was the method of Sicard and Reilly,[28] consisting in the subdural injection of 5 c.c. of 0.5 per cent. saline or 0.0001 gm. mercury cyanid. Viton[29] thought that the injection of a mixture of this amount of cyanid with 0.015 gm. of novocain in 2 c.c. of saline was helpful in the treatment of tabes. Stillman and Swift,[30] however, claim that the subdural injection of normal salt solution, normal serum, serum arsphenamized *in vivo* or weak solutions of cyanid of mercury do not demonstrably increase the permeability of the spinal cord or brain for arsphenamin circulating in the blood at the time of the subdural injection. But subsequent investigations in syphilis, as well as in poliomyelitis, have shown that intraspinal injections of normal or medicated serum or saline solution does tend to increase extravasation from the vessels of the meninges or excretion by the choroid plexus, as shown by the experiments of Sekiguchi and myself,[31] and others. Shera[32] has recently advocated the intraspinal injection of 20 to 30 c.c. of 40- to 70-per cent. dilutions of the patient's own non-medicated serum for this purpose, but these precedures may also favor infection of these tissues with *Sp. pallida*, which constitutes the reason why most syphilologists are opposed to even simple spinal puncture during the early stages of syphilis until after sufficient treatment has been given to sterilize the blood. But whether or not such measures are worth while in the treatment of neurosyphilis, purely from the standpoint of increasing permeability for arsenic in the blood stream, is another matter. Keidel and

Moore,[33] for example, treated 25 cases of neurosyphilis with 6 weekly intraspinal injections of mercurialized serum (Byrnes method) followed twenty-four hours later by intravenous injections of arsphenamin to obtain maximum concentrations of arsenic in the blood at the time of maximum meningeal irritations, but found the results inferior to the Swift-Ellis method and an indication, in their opinion, that the mode of action of intraspinal medication does not depend upon increased permeability by the production of aseptic meningitis.

In 1916, Gilpin and Earley[34] advocated thorough spinal drainage as a means of favoring penetration of the tissues of the central nervous system during systemic treatment with arsphenamin or mercury by producing sudden reduction of spinal fluid pressure with resulting hyperemia and increased production of fluid; Dercum[35] became an enthusiastic advocate of the method and Finlaysen,[36] Hearn,[37] Schaller and Mehrtens,[38] Craig and Chaney,[39] Harrison[40] and others have observed favorable effects in some cases, but Pilsbury,[41] Fordyce,[42] Stokes and Osborne[43] and others have failed to secure encouraging or satisfactory results and believe the method less satisfactory than the Swift-Ellis method of intraspinal medication. My own experience has been apparently similar to that of Solomon,[44] who found spinal drainage of some value. It is exceedingly difficult, however, to evaluate the results in definite terms and in the final analysis I believe it is inferior to methods employing intraspinal injections of medicated serum, although I believe that spinal drainage as practiced by Dercum may increase permeability for the organic arsenicals in some cases of neurosyphilis.

A promising work along these lines at present is the method of Corbus, O'Connor, Lincoln and Gardner,[45] who gave an intravenous injection of 100 c.c. of a 15-per cent. solution of sodium chlorid; this is followed by an enormous drop in spinal fluid pressure and the patient is kept in bed. About six hours later 0.6 to 0.9 gm. of neoarsphenamin is injected intravenously at a time when the spinal fluid pressure is being restored and a few hours later the patient is allowed to get up. In 26 out of 28 cases of neurosyphilis this was followed by an increase of arsenic in the spinal fluid. The method is based upon the work of Weed and McKibben,[46] who found that hypertonic sodium chlorid solutions given intravenously or solutions of sodium sulphate and sodium chloride administered by rectum lower intracranial tension with consequent pressure changes in the cerebrospinal fluid. Their findings were corroborated by Foley and Putman[47] and the phenomenon has since had practical application in the field of surgery. Fordyce, Rosen and Myers,[48] in an analysis of 76 specimens of cerebrospinal fluid for arsenic at various periods, after the application of this method, found a considerable regularity in the quantity of arsenic penetrating to the cerebrospinal fluid. Only one specimen was found in which it was not present. While there appears to be a uniformity in penetration, they conclude that it would seem from the data thus far collected that this procedure does not favor the passage of large amounts. What may be called the reverse of this mechanism has been attempted by Wynn,[49] who tried the effects of giving an intravenous injection of 200 c.c. of 15 per cent. sodium chlorid solution after intraspinal medication, in order to promote absorption of the drug from the subarachanoid space; the method however, has not yielded encouraging results since some cases were aggravated and others not improved.

But far more effort has been expended in attempts to augment the effects of systemic medication in neurosyphilis, by various methods of intraspinal medication. Before taking up a consideration of this subject, however, it

may be well to express an estimate of the value of systemic medication alone with various spirocheticidal medicaments.

Value of Arsphenamin, Silver Arsphenamin and Neoarsphenamin in the Systemic Treatment of Neurosyphilis.—Both arsphenamin and neoarsphenamin by intravenous injection have been proven to possess a high degree of curative activity in neurosyphilis; silver arsphenamin has been especially recommended by Stewart[50] and others. In the early types they appear sufficient when administered in conjunction with mercury and the iodids. Many physicians are of the opinion that the intravenous injection of arsphenamin is the more curative phase of the Swift-Ellis method; Sachs,[51] Kaliski and Strauss[52] are especially convinced of this and practically all syphilologists are agreed that intraspinal medication should be deferred in any given case until intravenous arsenical treatment associated with mercury by inunction or injection and iodids by ingestion, have been given a thorough trial. It is admitted that large doses are required, as likewise treatment over a long period of time in order to secure therapeutic results.

Arsphenamin is superior to neoarsphenamin in the treatment of neurosyphilis; it appears to possess a higher degree of penetrability for the tissues of the central nervous system and of the two compounds, is the drug of choice. Silver arsphenamin is regarded by some physicians as slightly superior to arsphenamin, but this has not been proved and in my opinion it is neither superior nor to be preferred. Neoarsphenamin is quite sufficient for the treatment of early, spinal fluid or asymptomatic neurosyphilis, but for symptomatic cases, arsphenamin is to be preferred. Sicard,[53] however, claims to have obtained noteworthy results in the treatment of tabes, paresis, syphilitic paraplegia and hemiplegia by the daily administration of neoarsphenamin subcutaneously or intravenously in doses not exceeding 0.15 gm. over an extended period. He has given as much as 50 gm. within a space of two years. He believes this method of treatment to be less dangerous than weekly injections of larger doses and superior in its results in nervous syphilis and in its effect on the Wassermann test in the blood and spinal fluid. He has not observed any nitritoid reactions, but mild erythemas have been observed in 6 to 8 per cent. of cases, benign icterus in about 2 per cent. and progressive diminution of the ankle jerks in about 60 per cent. of cases. Mott[54] mentions this "driblet method" quite favorably, administering the drug in glucose solution by subcutaneous injection. Arata[55] has also rendered a favorable report on the method. As in the treatment of chronic cardiovascular syphilis, it is certain that prolonged administration is required in conjunction with mercury and iodids, for securing a cessation of progressive infection of the tissues and especially of parenchymatous infections.

Value of Sulpharsphenamin and Tryparsamid in the Systemic Treatment of Neurosyphilis.—Considerable interest is being shown at present in sulpharsphenamin and tryparsamid in the treatment of neurosyphilis. Voegthin, Smith, Dyer and Thompson[56] have found that both drugs possess a higher penetrability for the central nervous system than arsphenanim, silver arsphenamin and neoarsphenamin. Tixier and Duval[57] have reported excellent results with small doses of sulfarsenol (starting with 0.06 gm.) by daily subcutaneous injection after Sicard's method described above, when used in conjunction with mercury and iodid. Stokes and Behn[58] have had a particularly favorable experience with sulpharsphenamin in the treatment of neurosyphilis following courses of light weekly intramuscular injections of 0.4 gm. (0.2 gm. in each buttock), and their report warrants a thorough trial of the drug, although it is still too early to express a final opinion of its value and

especially in the treatment of tabes dorsalis and paresis. Just why sulpharsphenamin should have given them exceptionally good results is difficult to state, since the chemistry of the compound does not indicate that it is much different from neoarsphenamin insofar as lipoid solubility is concerned while its spirocheticidal activity is not greatly and indeed, not usually as high. In my opinion, the alleged superior results are probably due to the method of administration, since intramuscular injections are followed by slower absorption and slower elimination, the latter being the chief factor and enabling a better penetration by the longer time afforded for contact with the tissues, which appears to be a matter of great importance in the penetration of the parenchyma of both brain and cord by the organic arsenicals. I do not doubt that, if arsphenamin and neoarsphenamin could be administered in similar doses by subcutaneous or intramuscular injection, their therapeutic properties in neurosyphilis would be greatly enhanced; indeed, this is indicated both by the results of animal experiments and Sicard's success with daily small doses of neoarsphenamin by subcutaneous injection.

Probably the same factors explain the apparently excellent results being reported from intravenous injections of tryparsamid combined with intramuscular injections of mercury, in the treatment of paresis, tabes and other types of chronic neurosyphilis. This compound is very much lower in spirocheticidal activity than arsphenamin, neoarsphenamin or sulpharsphenamin, as shown in Chapter XXIII, but it is likewise less toxic for body cells permitting the intravenous injections of doses as high as 3 gm. once a week or two weeks for courses of ten or more injections. This brings a very large amount of pentavalent arsenic to the tissues at one time with apparently a high degree of penetration, since the compound is eliminated somewhat less rapidly. Aside from this it would appear, from the investigation of Voegtlin and his associates, that pentavalent organic arsenicals possess in high degree the power of penetration of the tissues of the brain and cord, as shown by the results observed in the treatment of trypanosomiasis of the subarachanoid space of rabbits with tryparsamid and the sodium salt of 3-amino-4-oxy-phenol arsonic acid. But it is curious that tryparsamid even in doses as high as 3 gm. for adults, possesses so little spirocheticidal activity in early syphilis that it is very much inferior to arsphenamin, neoarsphenamin and sulpharsphenamin in the treatment of primary and secondary syphilis and early asymptomatic neurosyphilis; its value is confined largely and almost entirely to the treatment of chronic types of neurosyphilis as shown by the reports of Lorenz and his colleagues,[59] Moore, Robinson and Keidel,[60] Hindman,[61] Solomon and Viets,[62] Schaller,[63] Stokes,[64] Cady,[65] Skoog,[66] Read,[67] Neyman,[68] Kennedy and Davis,[69] Wile,[70] Crawford,[71] Vines[11] and others.

Moore and his colleagues have emphasized that the drug should not be used in cases with optic nerve involvement and in a subsequent study by Woods and Moore[73] of 241 patients receiving more than 3000 injections of the compound, dimness of vision was observed in 10.2 per cent. and constriction of the visual fields for form, without scotomas, and with or without diminution in visual acuity, in 5.5 per cent. In their experience they found (1) that 94 per cent. of all reactions occurred early in the course of the treatment, by the time of the tenth injection; (2) that visual disturbances bear no direct relationship, within certain limits, to the dose of the drug administered; (3) that disease of the central nervous system, especially general paralytic and tabetic neurosyphilis, is to some extent a predisposing factor to visual disturbance from tryparsamid; (4) that preexisting syphilitic disease of the optic nerve or retina is not necessarily a contraindication to

the use of tryparsamid; (5) that it is probable that the drug exercises, in some instances, a direct toxic action on the retina or the optic nerve; (6) that before beginning tryparsamid therapy, every patient should be examined by a competent ophthalmologist as to visual acuity, fundi and visual fields. The occurrence of any visual disturbance must form the occasion for a second examination. Lillie,[74] however, in a study of the eyes of 114 cases of syphilis under treatment with tryparsamid, found that ocular changes occurred as often with arsphenamin treatment as with tryparsamid and he is inclined to believe that the changes in vision, fundi and perimetric fields are due rather to syphilis than to the drugs.

It would appear, therefore, that the preliminary reports on tryparsamid in late neurosyphilis and especially paresis, tabes and taboparesis are encouraging; all are agreed, however, that it is too early to express a final opinion since the improved patients must be observed for at least five years to determine the length of the remissions, since no one can assume at present that the cases are actually cured in the sense of complete extirpation of the disease. Tryparsamid is also of value for its roborant and nutritional effects but must be given with great care when eye lesions are present even though these do not absolutely contraindicate the use of the drug. But its employment should be confined to late neurosyphilis as it is inferior to arsphenamin and its substitutes in the treatment of early syphilis and also in late syphilis of organs other than of the central nervous system.

Value of Mercury, Bismuth and the Iodids in the Systemic Treatment of Neurosyphilis.—Of course, mercury has been for ages the chief drug for the treatment of neurosyphilis and at the present time practically all neurologists and psychiatrists advocate its administration along with the organic arsenical compounds. Mercury, however, has not produced any noteworthy results in paresis, although it is not to be overlooked that the remissions of this disease under the older forms of mercurial treatment and sometimes lasting several years, may have been due in part to its therapeutic effects. Mercury, and especially by inunction, is particularly valuable in the treatment of tabes dorsalis, and in my opinion should never be omitted in this disease, especially if treatment can be conducted by inunction after the methods practiced at Aachen abroad and the Hot Springs of America. In early asymptomatic neurosyphilis, dependence should not be placed in mercury alone because it is decidedly inferior in spirocheticidal activity to arsphenamin and its substitutes, but in late neurosyphilis of all types, it deserves a prominent place in treatment and especially by inunction. Just why mercury has failed in paresis is unknown; it is my opinion, however, that the metal is lacking in penetrating properties for the parenchyma of the brain and fails to accumulate in the brain to spirocheticidal concentrations, although it may do so inthe meninges on account of their fibrous structure and therefore prove more valuable in the treatment of meningeal tabes and meningovascular lesions than in parenchymatous infections.

Bismuth is apparently proving more useful than mercury and especially in meningeal types of neurosyphilis with headache. Favorable reports have been rendered by Shivers, Dessert, Hudelo, as well as by Milian,[75] Marie and Fourcade,[76] Tixier,[77] Foster and Smith[78] and others, although it is too early to express a final opinion of the value of bismuth salts in neurosyphilis. I believe, however, that present results justify one using the insoluble compounds by intramuscular injection as a substitute for mercury and especially when injections of the latter are contra-indicated.

The iodids are of most value in chronic or late neurosyphilis for their dissolving and absorbing effects upon syphilitic exudates and fibrous tissue.

For this reason they are especially indicated in tabes dorsalis but probably also possess some value in paresis and other late lesions although there are no special indications for their administration in early asymptomatic neurosyphilis. Oral administration is apparently sufficient for obtaining their beneficial effects, although several reports indicate that intravenous injections of sodium iodid may be especially serviceable in tabes and paresis.

Practical Value of Systemic Treatment Alone in Neurosyphilis.—It is apparent, therefore, that a good deal may be done therapeutically for many neurosyphilitics by systemic medication alone without resorting to spinal drainage, intraspinal or intraventricular medication, etc. This is especially true in early asymptomatic neurosyphilis and in later meningeal and meningovascular types. Gennerich[79] however, resorts to intraspinal medication almost at once and believes that best results are observed when combined intravenous and intraspinal treatment are given without delay, but I believe that Fordyce and his colleagues give systemic medication alone fair trial in the majority of cases of neurosyphilis before resorting to the Swift-Ellis treatment, unless their cases have already had considerable systemic treatment without satisfactory results before coming under their care. Now that sulpharsphenamin and tryparsamid have apparently increased the efficacy of systemic treatment, it would appear especially indicated to first try thorough treatment with the organic arsenicals combined with mercury or bismuth and the iodids, resorting to intraspinal or intraventricular medication only when these fail to arrest the disease.

Intracarotid Injections in the Treatment of Paresis.—In relation to this subject special mention may be made of recent attempts to treat paresis by injecting neoarsphenamin and sulpharsphenamin directly into the carotid artery in the treatment of paresis since it would appear plausible that by this route the drugs would reach the diseased areas more directly and in higher concentration than by other methods of administration. Knauer[80] apparently used this method in 1919 and Endeslin and Justi[81] devised a method for giving the injections directly through the skin without exposure of the artery. Recently Hirsh, Myerson and Halloran[82] have described their technic and experiments along with a preliminary report on the results of approximately 250 injections of sulpharsphenamin and tryparsamid with improvement in some cases and the opinion that the procedure is warranted in the treatment of a larger series of cases.

Intraspinal Treatment of Neurosyphilis.—In view of the generally unsatisfactory results of systemic treatment of paresis with arsphenamin and mercury and the success attending the treatment of tetanus with intraspinal injections of antitetanus serum and epidemic cerebrospinal meningitis by intraspinal injections of antimeningococcus serum, it is not surprising that early and persistent attempts were made to improve the treatment of neurosyphilis by introducing spirocheticidal substances directly into the spinal fluid with the hope that they would not only reach spirochetes in the meninges but likewise those organisms entrenched in the deeper portions of the brain and cord. The first attempts in this field were made by Marinesco[83] in 1911 followed independently and almost simultaneously by Swift and Ellis[84] and Wechselmann.[85] Since then many different methods have been advocated and a large literature accumulated, most of which is of a controversial and confusing character on the merits or demerits of this or that method or on the subject of intraspinal *versus* systemic treatment in general. Before, however, taking up the questions of the practical value of intraspinal treatment in general, the indications, contraindications and probably mechanism of curative or beneficial activity, I shall briefly review the status of various agents employed and of methods for their administration.

Subarachanoid Injections of Neoarsphenamin in the Treatment of Neurosyphilis.—Wechselmann was the first to inject dilute solutions of neoarsphena-

min intraspinally and the method received enthusiastic support from Ravaut[86] in Europe and Wile[87] in America. As a general rule, from 0.0005 to 0.005 gm. were injected, but it was not long before the method received unfavorable mention by reason of numerous and sometimes serious evidences of meningeal and cord irritations. Schubert[88] then devised a plan for dissolving 0.045 gm. of neoarsphenamin in 3 c.c. of spinal fluid and mixing 0.1 or 0.2 c.c. with 5 c.c. of fluid which had been allowed to flow into a funnel attached to the lumbar puncture needle. This method undoubtedly reduced the incidence and severity of cord and meningeal irritations and the principle has since been adopted for the Swift-Ellis and Olgilvie methods; Gennerich[89] especially found the method superior to neoarsphenamin dissolved in water or saline solution. But at the present time methods of these kinds are not generally employed because of the reports by Corbus,[90] Gordon,[91] Sachs, Strauss and Kaliski[92] and others indicating that severe local reactions may occur. The milder form of these consist of urinary retention, and incontinence, rectal paralysis, parethesias and numbness of the buttocks and legs, ataxia in the legs, girdle pains, trophic ulcers, and in severe cases complete paralyses and death. If given at all, the dose should not be more than 0.0005 gm. in spinal fluid; arsphenamin is so much more irritating that its use by this method is altogether out of the question unless the dose is much smaller.

Subarachanoid Injections of Serum Arsphenamized and Neoarsphenamized in Vivo and in Vitro in the Treatment of Neurosyphilis; the Swift-Ellis and Ogilvie Methods.—In order to prevent this irritation of the meninges and cord by the medicament, Swift and Ellis devised their method of arsphenamizing the serum *in vivo* by injecting arsphenamin intravenously and drawing off 40 to 50 c.c. of blood about one hour later from which the serum was secured. Since then the method has been slightly modified in that the blood is drawn off one half hour after injection and the serum mixed with spinal fluid allowed to flow into the barrel of a syringe attached to the spinal puncture needle, as described on page 858, before injection. It is the one method that has stood numerous attacks upon intraspinal therapy in general and undoubtedly is the best and safest method in use today for *repeated* intraspinal injections. Marie and Levaditi[93] have suggested injecting arsphenamin into the lower animals and using their serum, but the method is mentioned only to be condemned since the serum is in no wise less irritating, produces allergic sensitization and denies the patient the very considerable benefit of the intravenous injection, which constitutes in no small degree the value of the Swift-Ellis method.

Ogilvie,[94] however, soon suggested a useful modification to meet the objection of uncertain dosage of arsphenamin in the Swift-Ellis serum, by adding arsphenamin to the serum *in vitro* followed by heating, etc., as described on page 872. It was soon found however, by Draper[95] and others, that the serum sometimes produced irritation, and Fordyce[96] warned against using more than 0.0005 gm. as a dose. Marinesco and Minea[97] then advocated a method of adding 0.006 to as much as 0.012 gm. of neoarsphenamin to serum *in vitro*, but subsequent investigators found these amounts too irritating so that not more than 0.0005 to 0.001 gm. is employed at present. In my opinion the addition of arsphenamin or neoarsphenamin to serum *in vitro* aids in increasing its curative activity and, I believe, that a combination of the Swift-Ellis and Ogilvie-Marinesco-Minea methods is always worthy of trial, to be abandoned, however, in favor of the straight Swift-Ellis method if even minor signs of irritation develop. But the dose of arsphenamin in alkalinized solution to be added to serum should not exceed 0.0005 gm. and the dose of neoarsphenamin should not ordinarily exceed 0.001 gm. in the

interest of safety and especially if a series of injections are to be given. During the past three years I have injected 0.9 gm. neoarsphenamin intravenously, drawing blood a few minutes later and adding 0.001 gm. of neoarsphenamin to the serum, as described on page 872.

Subarachanoid Injections of Mercury and Mercurialized Serum in the Treatment of Neurosyphilis.—I have already mentioned Sicard's method of injecting 0.1 mg. of mercuric cyanid, but this plan has long since been abandoned as likewise Horsley's[98] subarachanoid lavage with mercuric chlorid. Byrnes,[99] however, has suggested a much more useful method consisting of an albuminate of mercury prepared by mixing 1.3 to 2.6 mg. of mercuric chlorid with 12 c.c. of human serum (not necessarily the patient's own), adding 18 c.c. of normal saline and heating at 56°C. as described on page 890. Horse serum should not be employed because of allergic sensitization. When the serum is mixed with spinal fluid before injection, it produces none or but slightly more irritation than observed in the Olgivie method and has been favorably reported upon by Hunt,[100] Haller,[101] Nicholson[102] and others from this standpoint as well as because of greater ease of preparing and preserving the serum as compared with arsphenaminized or neoarsphenaminized serum, whereas Hazen[103] and others state that too much irritation is produced.

Routes of Subarachanoid Injection in the Treatment of Neurosyphilis.—In Chapter LXI, I have described three routes of injection: intraspinal, intraventricular and intracisternal, and this phase of the subject has not received by any means the attention it deserves. Owing to the greater ease of intraspinal injections (lumbar region), this method has become the most popular and widely employed.

In 1914, Berriet[103] injected arsphenamized serum through the sphenoidal fissure into the large basal subarachanoid cistern and recently McCusker,[104] Ebaugh[105] and others have advocated injections of arsphenamized or neoarsphenamized serum into the cisterna magna by Ayer's technic. It is stated that by this route the serum is brought nearer to the seat of cerebral syphilis, that it is just as safe and free of reactions as intraspinal injections and more practical than intraventricular injections.

The latter was apparently first practiced by Ballance[106] in 1914, who injected as much as 90 c.c. of arsphenamized serum into the lateral ventricles by passing a blunt trocar and cannula through a trephine opening and the cerebral cortex. Since then Hammond, Sharpe and Smith,[107] Knapp,[108] and Riggs[109] have described methods and apparently encouraging results in the treatment of paresis.

The use of lumbar puncture is based largely upon the assumption that serum injected in this locality is quickly diffused upward over the upper cord and cerebrum, as observed in the experiments of Dandy and Blackfan[110] with phenolsulphonephthalein, India ink and dyes. But from my experiments with rabbits, I very much doubt if diffusion occurs to the extent indicated by these investigations. Insofar as lumbar injections of serum colored with dyes are concerned, the injection of rabbits with 0.5 c.c. per 1500 grams of weight have almost never shown macroscopic evidences of diffusion above the level of the medulla whereas intracistern injections have shown diffusion downward and over the base of the brain while injections into either of the lateral ventricles have shown intense staining of both hemispheres of cerebrum, the base and diffusion downward as far as the upper third of the cord. Indeed, I have been so much impressed with the uniformity of these results that I suspect that the failure of intraspinal medication in the treatment of basilar meningitis and especially of paresis, is largely due to failure of

diffusion from the lumbar region to these structures whereas in early tabes excellent results have been observed because of the readiness of contact with the areas of meningitis involving the posterior roots and ganglia of the sensory nerves. I surmise that Solomon[111] is very much of the same opinion and I believe that intracistern and intraventricular medication are deserving of more serious attention and use in the treatment of paresis than they have had in the past, although with less serious types of neurosyphilis intraspinal medication apparently suffices.

Practical Value, Indications and Contra-indications of Subarachanoid Medication in Neurosyphilis.—It is hardly profitable for me to review here in detail the large literature upon this subject, as I believe it has been covered by the frank and complete discussion by Fordyce,[112] who recently reviewed his experience of the past ten years with special reference to the value, indications and contra-indications of the Swift-Ellis method.

Possibly Fordyce has ascribed more benefit to the intraspinal than to the intravenous phase of the Swift-Ellis method; at least the opponents of the method are of this opinion and there can be no doubt of the value of systemic treatment alone, but when the latter has been tried and failed to at least arrest the progress of infection, I believe it is our duty to employ intraspinal medication unless there are distinct contra-indications. The latter, in my practice, have been present more in cases of tabes than in paresis or other forms of neurosyphilis, in the form of rectal and bladder disturbances which are so easily intensified by lower cord irritation. When in doubt about the wisdom of employing intraspinal medication in cases in whom it is particularly imperative to minimize all meningeal and cord irritation, I believe the Swift-Ellis method is by all odds the method of choice; certainly we should avoid overdosage and injurious reactions as emphasized by Fuchs[113] and others.

Mechanism of Therapeutic Activity of Subarachanoid Medication in Neurosyphilis.—If benefits are to be ascribed to subarachanoid medication, how are they to be explained? Failure to provide an acceptable explanation of the mechanism has alone brought undeserved criticism of the Swift-Ellis method.

That some medicament is contained in the serum taken from blood drawn within half an hour after the intravenous injection of 0.6 gm. arsphenamin or 0.9 gm. of neoarsphenamin is certain, even though the results of analyses in terms of metallic arsenic have shown only traces with a portion retained in the clots. The fact that the serum increases in spirocheticidal activity as a result of heating at 55°C. before injection strongly suggests that this is due to oxidation of the arsphenamin or neoarsphenamin with the production of the highly spirocheticidal trivalent oxids of which "arsenoxid" is the best known; at least I am unable to offer any other explanation for the phenomenon, which appears to occur as a result of heating the serum. But is the amount present sufficient for spirocheticidal activity and especially when it is remembered that the serum is still further diluted by the cerebrospinal fluid after injection? Swift and Ellis[114] claim to have demonstrated the spirocheticidal activity of the serum *in vitro* and Stuhmer[115] states that it has a similar action on trypanosomes. Unfortunately my own experiments have not yielded conclusive results. I have repeatedly mixed equal parts of a saline-rabbit-testicular-syphiloma-tissue suspension of virulent *Sp. pallida* with patient's serum drawn immediately before, one half and one hour after intravenous injections of arsphenamin and neoarsphenamin, at 37°C. for intervals from five to fifteen minutes, followed by intratesticular inoculation of rabbits, as a test for spirocheticidal activity, with the result that the mixtures with serum drawn before injection were sometimes non-infectious when the mixtures with serums drawn after injection were not, indicating an apparently enhanced spirocheticidal activity of the Swift-Ellis serums. However, the serums before and after injection were both spirocheticidal in some tests while in other tests they were not; in other words *Sp. pallida* is rather easily destroyed at 37°C. in fifteen minutes and the results were sufficiently irregular to prevent definite conclusions, but, in general terms, the results tend to confirm Swift's claims for increased spirocheticidal activity. Numerous experiments *in vitro* with saline

suspensions of cultures of *Sp. pallida* have indicated in a general manner that concentrations of arsphenamin in the neighborhood of 1:500 to 1:1000 are required for the almost immediate destruction of the parasites, whereas in long contact over days or weeks concentrations of arsphenamin about 1:8000 and neoarsphenamin about 1:3000 are required, as discussed in Chapter XXVIII. It is certain that the concentrations in the Swift-Ellis serum are not ordinarily this high. Probably a dose of 0.6 gm. of arsphenamin per 70 kilograms of weight gives a dilution of not less than 1:10,000 when the volume of blood and other body fluids as well as rapid fixation by the organs, are taken into consideration. But when one recollects the wonderful and prompt disappearance of spirochetes from a chancre following the intravenous injection of 0.010 gm. of arsphenamin per kilogram of weight of either human being or rabbit, and what a high dilution this represents in the blood and lymph, I believe that most will agree with me that it is impossible to accurately correlate the results of spirocheticidal activity *in vivo* and *in vitro* and especially since so many other factors require consideration in the former. Therefore, while Swift-Ellis serum may be sufficiently spirocheticidal in the subarachanoid space, there are reasons for being much surer of this when 0.0005 gm. of arsphenamin or 0.001 gm. of neoarsphenamin are added *in vitro*, as in Ogilivie's and my methods, although the total amounts are still below what the results of *in vitro* spirocheticidal tests would lead one to expect as required for spirocheticidal effects in the subarachanoid space after dilution with 50 to 150 c.c. of cerebrospinal fluid. Certainly not all or even a goodly proportion of the beneficial results, are to be ascribed to this one factor; other agencies must be concerned.

Very probably considerable benefit accrues from the passage of the medicament from the blood into the tissues of the central nervous system and especially when it is held in relatively prolonged contact with them by intramuscular injection or intravenous injection over prolonged periods. Furthermore, I believe it is settled as an established fact that in the majority of cases of neurosyphilis, traces of the arsenicals reach the spinal fluid after intravenous injection, probably even in spirocheticidal concentrations in some cases, from which a degree of therapeutic effect is to be expected and especially in meningeal, vascular and meningo-vascular infections. Furthermore, that which has passed into the spinal fluid is again absorbed with possible spirocheticidal effects in passage, since absorption of the organic arsenicals introduced directly into the subarachanoid space is shown by the production of dermatitis, etc., as reported by Schönfield[116] with silver arsphenamin, in the course of a very interesting series of experiments upon the permeability of the choroid flexus and meningitis for argoflavin, indigocarmin, uranin and methylene-blue. When medicated serum is injected into the subarachanoid space, it is almost certain that a reaction follows of which increased congestion of the pia and possibly slight cellular and serous exudation are the chief changes; these alone are probably of therapeutic benefit, purely from the standpoint of a non-specific reaction to be discussed shortly, even though it were granted that they do not increase the penetration of spirocheticides present in the blood stream, which I believe actually occurs in some cases. It may be that the results of intraspinal treatment with the Ogilvie method, in which intravenous injections are not given, are as good as in the Swift-Ellis method in which intravenous injections are given, in which case the question of the importance of increased permeation of drugs from the blood is minimized. This is true, insofar as intravenous injections of arsphenamin are concerned in immediate relation with the technic, but examination of published records show that most cases treated by the Olgivie method have been under mercury or arsphenamin treatment at the same time, in which case the intraspinal injections may still encourage passage of the drugs into the tissues of the brain and spinal cord and especially mercury which is not eliminated as rapidly as the organic arsenicals.

Is a part of the curative effect of Swift-Ellis serum due to the presence of antibodies? I doubt that such is the case. The serum does not contain complement, because this is destroyed during the process of heating, although

following the injection, it is highly probable that complement from the patient's own blood reaches the spinal fluid as a result of the aseptic irritation and indeed, in cases of paresis at least, may be already in the spinal fluid along with natural antisheep hemolysin, as a result of increased permeability of the meninges in this disease (Weil-Kafka reaction). It is probable that the serum of the patient may contain small amounts of spirocheticidal substances, as indicated by the results of Eberson's[117] experiments, although the experiments of Broadwell and myself[118] yielded negative results with cultures of *Sp. pallida*. But granting that minute amounts of specific antibodies are present, and more cannot be granted, it is highly probable that after dilution with spinal fluid they play no important part in curative activity and especially since equally good results have been observed when the serum of perfectly normal non-syphilitic individuals have been substituted for patient's serum.

Probably an important effect of subarachanoid medication in neurosyphilis is to be ascribed to a non-specific protein reaction. It is true that if such occurs, the reaction must be mild in view of the fact that temperature and leukocytic changes do not occur at all or but to a slight degree. But the therapeutic activity of auto- and homo-serum therapy has been observed so frequently in the treatment of disease and especially of various diseases of the skin, that it is not unreasonable to assign a goodly proportion of the beneficial effects of subarachanoid therapy with plain or medicated human serum to this category and especially since it has been shown that the administration of non-specific protein agents has favorably influenced other chronic syphilitic lesions, as discussed in Chapter LXVIII. Indeed, as I shall shortly review, the subcutaneous or intramuscular injection of tuberculin, various vaccines, milk, etc., are reported to favorably influence neurosyphilis and it may be expected that subarachanoid injections of serum may have the same effects since absorption is rapid, yielding mild systemic reactions in addition to the far more important local reaction of which one important phase is hyperemia, duplicating in this respect the curative activity assigned to Bier's hyperemic treatment of chronic infections. It is not surprising, therefore, that Swift[119] and others have found that intraspinal injections of normal serum were beneficial even though it may not be granted that they increase the permeability of the choroid-meningeal complex for spirocheticides in the blood.

Nor can definite statements be made at this time of the mechanism of curative activity concerned in such non-specific reactions. Doubtless the increased congestion and positive polymorphonuclear leukocyte chemotaxis bring out in maximum degree the antispirochetic properties of the blood and may stimulate the tissues in contact with the spirochetes to produce increased amounts of specific anti-spirochetic substances, since we may accept as proven that the tissues in contact with *Sp. pallida* are constantly offering resistance which is frequently sufficient for holding the parasites in check or latency. Indeed, Gooken[120] has advocated injecting intravenously the spinal fluid removed in the course of intraspinal medication, with the idea in mind of giving the patient the benefit of any antibodies contained in the fluid, but unfortunately our information on the subject of antibodies in syphilis is so meager at present that one cannot discuss this phase of the subject with assurance, except to state that in all probability the antibody concerned in the Wassermann reaction is not in itself anti-spirocheticidal.

Before taking up the further discussion of non-specific protein therapy in the treatment of neurosyphilis, I believe that the mechanism of subarachanoid therapy may be summarized as follows, the relative importance of the various agencies being arranged in this order: (1) The non-specific effects

due to subarachanoid irritation following the injection of plain or medicated human serum from the patient or some other human being; this, in my opinion, is the most important reaction of all. (2) The increased penetration of the tissues by spirocheticides in the blood, as the result of disturbances of the choroid-meningeal complex due to spinal drainage and intraspinal injections. (3) The spirocheticidal activity of medicaments in the serum and (4) possibly, the influence of specific anti-spirochetic antibodies when autoserum medication is employed, as in the Swift-Ellis method. In order to secure the operation of all these agencies, I believe that subarachanoid medication is best conducted by a method combining the Swift-Ellis-Ogilvie methods, as my own does, being careful, however, not to add ordinarily more than 0.001 gm. of neoarsphenamin or arsphenamin to the serum *in vitro* in order to avoid too much meningeal and cord reaction with temporary motor and sensory disturbances.

Non-specific Protein Treatment of Neurosyphilis.—If the benefits following subarachanoid medication in neurosyphilis with plain or medicated serums are partly due to local and systemic non-specific reactions, which I believe are important agencies concerned in the therapeutic mechanism, of what value are subcutaneous or intramuscular injections of other protein agents, as milk and vaccines? I have already discussed the subject of non-specific protein therapy in the general treatment of syphilis in Chapter LXVIII, but it is so important in relation to the treatment of neurosyphilis, that additional mention of it would appear warranted.

Since Marro,[121] v. Halban[122] and numerous other clinicians had noted the apparent beneficial effects of malaria, typhoid fever, erysipelas, etc., upon the course of paresis, the idea gained ground that the production of acute fever by other agents may be beneficial in the treatment of paresis and tabes, since no one at that time had the temerity to produce infection for this purpose, as V. Jauregg-Wagner finally did some years later by inoculating with tertian malarial parasites, although Meyer[123] in 1877 had advocated the production of abscesses for this purpose.

On this basis V. Wagner[124] began using subcutaneous injections of pyocyaneus vaccine and then Koch's old tuberculin with encouraging results Pilcz[125] continued his work with tuberculin, starting treatment with 0.01 gm. and continuing up to 0.5 gm. and in 1911 reported that of a series of 86 cases of paresis treated in this manner, along with mercury and the iodids, about 60 per cent. showed improvement with marked relapses in 26 per cent.

At about the same time, Fisher[126] and Donath[127] began using subcutaneous injections of sodium nucleinate in doses of 0.5 gm. to as much as 3 gms. at weekly intervals along with the administration of mercury and iodid in the treatment of paresis, the latter reporting marked improvement in 47 per cent. of cases. Hauber,[128] Szedlak[129] and others followed with favorable reports on combined treatment of paresis with nuclein and mercury and Schacherl[130] in the treatment of tabes with subcutaneous injections of 0.001 gm. of tuberculin and salicylate of mercury, but Hussels,[131] Plange,[132] Lowenstein,[123] Kleinberger,[134] Bowman,[135] Friedländer,[136] Plaut[137] and others have claimed negative results and even harmful reactions in some instances from injections of tuberculin, nuclein, typhoid vaccine by intravenous injection, staphylococcus and streptococcus vaccines, etc.

More recently, however, interest in this subject has been renewed by the observations of Müller,[138] Roper,[139] Fischer,[140] Starcke,[141] Ahlswede[142] and others indicating that intramuscular injections of sterile milk, "aolan," "pylogetan," etc., are of value, along with specific therapy, in the treatment of paresis, tabes and other types of neurosyphilis although it is very difficult to properly evaluate the results by reviewing their accounts, because mercury or arsphenamin were usually given at the same time. Marie and Cohen[143] have reported the results observed with more than ninety general paralysis cases in which bismuth associated with shock treatment was followed by pronounced remissions in over 50 per cent. while the mortality was much less than in control groups. Of the three forms of what they call "leukopyretotherapy" tested (parenteral injections of milk, sodium nucleinate, or tuberculin), tuberuclin proved the most effectual. The bismuth was injected the day following the tuberculin; the dose of the latter was regulated to induce fever and leukocytosis without too much reaction. From ten to twelve of these therapeutic shocks were generally given, at three or four day intervals as a rule. Enough work has been done to indicate that the fever, leukocytosis and possibly an increase in serum proteases and lipases, believed to follow the administration of so-called non-specific agents, may be distinctly

helpful in the treatment of paresis and tabes and especially when administered along with arsphenamin, bismuth, etc. Care must be exercised in the selection of patients and in dosage in order to produce slight fever with increase of blood leukocytes and platelets and the amount to inject as well as the length of intervals between injections, varies with individual cases and the agent employed. Care must be also exercised in avoiding too many reactions in order not to produce "proteinogenous tiring" and "proteinogenous cachexia." In my experience the weekly intramuscular injection of 4 to 6 c.c. of market milk, boiled for ten minutes and cooled, or 5 c.c. of the various commercial preparations of milk or milk proteins, for 6 to 8 injections, ordinarily suffice as a course of non-specific treatment in conjunction with mercury by inunction, iodids by ingestion and arsphenamin, neoarsphenamin, tryparsamid or bismuth by injection. If tryparsamid is employed, auxiliary injections of such proteins are not required because this drug frequently improves the nutrition in a remarkable manner with a gain in weight and improvement of anemic states.

Just how non-specific protein therapy may improve the treatment of paresis and tabes cannot be stated. Probably such vascular changes as hyperemia and increased capillary permeability, which are believed to be produced by these agents, aids in the penetration of syphilitic tissues by specific spirocheticides, and for this reason I believe that arsphenamin or one of its substitutes, mercury or bismuth, should be always administered along with or immediately following the protein agent. Probably the fever and leukocytosis are in some manner beneficial and especially since the inoculation of paretics with the plasmodia of tertian malaria is claimed to constitute a valuable treatment for paresis, which subject may now receive discussion.

Treatment of Neurosyphilis with Special Reference to Paresis by Infection with Malaria and Relapsing Fever.—At the present writing the treatment of neurosyphilis and especially paresis, by inoculation with tertian malaria or *Sp. recurrentis* (African relapsing fever) is the most important subject in this field and is attracting great attention and favorable comment in Europe and particularly in Germany and Austria. The method has also been tried in Sweden and England, but to a lesser extent in France and America.

As mentioned in the preceding section there are numerous references in the literature bearing upon the apparent benefits accruing to the syphilitic from intercurrent febrile infections. In addition to those cited above, Olgivie mentions that Baillarger, in 1849, reported a remarkable remission in a paretic following a series of furuncles; also that Nash, in 1870, reported the "recovery" of a case following an attack of small-pox and that Fritsch and Buss, in 1884, reported remissions following erysipelas and sloughing infections.

These observations, however, did not attract much attention until V. Jauregg-Wagner[144] suggested in 1918, a possible means of improving the method of treating paresis with injections of tuberculin and nuclein, by inoculating with malaria, believing that the disease was of such a fatal character that any procedure not in itself dangerous, was justifiable in an effort to check its progress and terrors. Muhlens, Weygandt, and Kirschbaum[145] took up the subject by treating a series of 33 cases with inoculations with the *Sp. obermeiereri* and malaria plasmodia; of these four died (but not directly from infection), twelve improved and five did not improve, the remaining twelve being under observation for too brief a time to report upon. Pagniez[146] also reported favorable results and the review by Raecke[147] appeared to establish the method as meriting a thorough trial. Since then numerous reports have appeared, the majority of writers being of the opinion that a distinct advance has been made in the treatment of paresis.

Choice of Infection.—The majority of investigators have employed inoculation with *tertian malaria;* a few have employed the spirillum of relapsing fever (*Sp. obermeieri*).

The former is preferred because much easier of cure when a sufficient number of paroxysms have occurred and the results appear to have been more pronounced and satisfactory as reported by Weichbrodt,[148] Weygandt[149] and others. Due care must be taken, however, against the inoculation of paretics hyperesensitive to quinin and it is well to apply a skin test or better still, to administer 5 grains of quinin as a test before deciding upon malarial therapy.

Great care must be exercised in the selection of a strain of malaria plasmodium, since infections with aestivo-autumnal parasites (tropical malaria) have proved fatal, as stated by Muehlens[150] and Plehn;[151] Marinesco and Draganescu[152] have recorded a fatal *plasmodium vivax* infection in a case of paresis activated by injections of arsphenamin. Wagner and others have shown that the experimental asexual tertian infections are much easier to cure with quinin than natural infections resulting from anopheles transmission and while a few investigators have reported remissions of the malaria, no particular difficulties in the cure of this infection have been reported.

Technic of Inoculation.—The malarial inoculations have been made by injecting patients subcutaneously with 1 to 4 c.c. of blood drawn from a malarial patient during or soon after a febrile attack. As a general rule, the strain is maintained by the successive inoculation of paretics and the treatment, therefore, is especially applicable for psychiatric or other institutions in which transmission can be maintained. Since the organisms cannot be successfully cultivated fresh blood should be obtained from an individual carrying the parasites but if obtained from a case of natural or mosquito infection, great care must be exercised to avoid quartan and aestivo-autumnal inoculations. It is far better to obtain blood from a paretic case if at all possible.

The patients must be carefully screened against mosquitoes and especially if anopheles are present in the locality, in order to guard against accidental transmission of malaria, although, as a matter of fact, it would appear from recent experiments in Europe, that the inoculated disease is not transmitted by anophelines as is the natural disease.

As a general rule, there is an incubation period of 10 to 16 days following subcutaneous inoculation before paroxysms develop; when the inoculation is made by intravenous injection, the incubation period is ordinarily about 4 to 8 days. About 10 per cent. of patients do not "take" on the first inoculation and must be re-injected.

The inoculation should be given only to individuals in the early stages of paresis in good general condition; advanced cases do not fare well on account of the debilitating effects of the 10 or more paroxysms required for success.

The same method may be employed for the transmission of relapsing fever or the strain may be maintained by inoculating mice and using saline emulsions of the brain for the subcutaneous inoculation of paretics, when the mice show large numbers of parasites in the peripheral blood. The ease of maintaining a strain in mice is a point distinctly in favor of this infection, but as stated above, the results do not appear to be as good as with malaria and more difficulty has been exercised in curing the infection with arsphenamin than in the cure of tertian malaria with quinin. When relapsing fever therapy is employed, it is necessary to guard against the possible accidental transmission of the disease by lice and bed-bugs.

Duration of Infection.—Most investigators have allowed their patients to have 8 to 15 paroxysms of malaria, but it is advisable to make daily examinations of the blood and interrupt the infection by the intramuscular or subcutaneous injection of quinin, if the malaria becomes too severe. Further indications for immediate discontinuation are sudden loss of strength, pronounced icterus and cardiac weakness.

Many different methods for curing the malaria have been advocated, as the intramuscular or subcutaneous injection of 1 gm. (15 grains) of quinin dihydrochlorid, quinin and urea hydrochlorid or quinin urethan, daily, for two or three days followed by 0.5 gm. on the fourth and sixth days; these are followed by 5 grains of quinin bisulphat daily for a week and these in turn by six to nine weekly intravenous injections of neoarsphenamin. Askgaard[153] gives 0.25 gm. of quinin by injection three times a day for 4 days; this is followed in order by 2 days interval, quinin for 10 days, an interval of one month, quinin for 3 days, an interval of 2 months, quinin for 3 days, an interval of 3 months and finally quinin for 3 days. Great care, however, must be taken to insure complete cure of the malarial infection; Kogerer[154] states that at the Vienna Psychiatric Institute, after tests of the blood had been negative following treatment of the induced malaria by quinin and neoarsphenamin, the blood is examined after roentgen irradiation of the spleen. Later, the use of injections of sodium nucleinate, 5 c.c. of a 10-per cent. solution, was found to be more efficacious for provoking latent malaria. In a series of 25 patients, 3 gave positive blood smears after this injection, and continued treatment of the experimental malaria was instituted. Muchlens states that patients should not leave the hospital until they have been free from parasites for at least three or four weeks and that those inoculated with relapsing fever should remain under observation for at least a month after the last attack. *As a general rule however, cure is easily effected.*

Relapsing fever therapy has been ordinarily allowed to run for periods of four months to two years; Sagel[155] reports one case under treatment for fifty months. The infection may be cured by the administration of arsphenamin or neoarsphenamin, as described in Chapter XXXI.

Indications for Malarial Therapy.—Most success has been observed in paresis and especially early cases. Even in advanced maniacal cases remissions have been produced, as I shall mention shortly in a brief review of the literature. The treatment has also been used in other types of neurosyphilis and especially in tabes dorsalis and some clinicians in Germany and Austria are so impressed with the success and safety of malarial therapy, that they are using it in early asymptomatic cases as a prophylactic measure.

Contra-indications and Dangers of Malarial Therapy.—When due care is exercised in the selection of patients and the malarial parasite (tertian only to be employed), the method is regarded as almost devoid of danger unless the patient is elderly and very debilitated; Loewenfeld regards cases of aortitis as poor risks. Quartan and aestivo-autumnal infections have proven very difficult to terminate and some have ended fatally. Too severe paroxysms should always be checked by quinin. It may be that the malarial attacks increase the danger of toxic reactions from arsphenamin, neoarsphenamin or their substitutes sometimes administered after the malarial infection has been terminated. I know of three fatalities following the administration of neoarsphenamin to paretics subjected to malarial therapy. It is known that malaria alters the usual pathological changes in paresis and I surmise may particularly alter the vascular changes and increase the vasculotoxicity of the arsenicals.

Combination Malarial and Chemotherapy of Paresis.—This naturally brings up the question of the dangers and advantages of combining malarial with the usual therapy of neurosyphilis. As I shall point out shortly the clinical results in malarial therapy have been more striking than the effects upon the Wassermann reaction and spinal fluid changes, although these may undergo improvement at the same time. But Scherber[156] and Scherber and Albrecht[157] believe that best results are secured by combining malarial with treatment by arsphenamin and its derivatives and mercury; at least the serological results appear to be better. But if much treatment is given I believe it well to guard the dosage carefully and when the arsenicals are given after a series of malarial paroxysms, it is advisable to begin with small doses and gradually increase.

Results Observed in the Treatment of Paresis and Other Types of Neurosyphilis.—Pilcz,[158] reporting on malarial therapy in 141 cases of paresis, states that 51 cases completely recovered; 18 showed marked and persisting remission without being able, however, to return to their former occupations, and 57 cases became stationary or showed an incomplete remission. Fifteen patients died but not of malarial infection. He points out that there is no parallelism in the degree and duration of the remissions, on the one hand, and in the serologic reactions, on the other. The best results are obtained in cases with maniacal symptoms and in cases of simple dementia; the hypochondriacal, presenile and catatonic forms of disease show a less favorable prognosis. Generally, remission does not immediately follow treatment; it develops gradually, finally becoming permanent. He states that the method is efficacious not only in cases of paresis, but also in tabes and other forms of neurosyphilis and that in disseminated sclerosis, also good results have been obtained.

Gerstmann[159] has reported that of 304 cases of paresis subjected to malarial therapy, 90 were not influenced, 102 had remissions of varying degree and duration while 112 were able to resume work although minor psychic signs were present. He states that the remissions did not begin ordinarily for several weeks after inoculation. No effect was noted upon the serology of the blood and spinal fluid. In some cases a marked change in the disease was observed and especially a change into peculiar paranoid forms with persecutory delusions and hallucinations of hearing.

Templeton[160] in a review of 296 cases of paresis subjected to malarial therapy, states that complete remissions occured in 112, although the duration of these is not stated in all cases; Grant[161] reports that the therapy was beneficial in all of a series of 40 cases. McAlister[162] states that of 9 cases under prolonged treatment and observation 2 were very much improved, 4

slightly and 3 not at all. Worster-Drought and Beccle[163] report that of 14 paretics subjected to malarial treatment, eleven being early, two advanced and one juvenile, 2 improved sufficiently to resume work, 4 had a definite remission and 8 only a slight remission or none at all. Grant and Silverton[164] state that in all of a series of 40 cases, improvement occurred in the serology of the serum, spinal fluid or both.

Plehn[165] reports that in at least one third of a series of 41 cases treated with malaria, an improvement of the mental psychic condition occurred although improvement of the somatic changes (pupils) was rare. No beneficial effects were observed however, in cases of tabes or other types of neurosyphilis. Additional reports have been made by MacBride and Templeton,[166] Graf,[167] Berger and Untersteiner,[168] Plehm,[169] Bunker and Kirby,[170] MacBride and Carmichael,[171] Grant,[172] Sagel,[173] and Herrmann.[174]

Fewer reports have been rendered on the results of relapsing fever therapy. Sagel[175] states that 11 out of a series of 17 cases of paresis were considerably improved. Weimer[176] reports that in a series of 9 cases of paresis and taboparesis, some improvement in psychic symptoms and serology occurred in the majority and certainly more than commonly observed under arsphenamin therapy alone and Artwinski[177] states that of 17 early cases, remissions have been produced in ten with improvement in the spinal fluid changes in five.

Of course it is not yet possible to express a definite opinion of the results, since more time is required for the final evaluation of these methods according to the duration of the remissions and their degree of symptomatic relief. Many of the German writers have used the word "cure" rather freely, but I believe this should be applied only in a relative sense to designate symptomatic improvement to the extent of restoration of working capacity, and not in the sense of complete extirpation of the infection. Even among those regarded as "cured" some psychic stigmata have usually persisted rendering a return of these patients to positions of responsibility hazardous and inadvisable. But it would appear that malarial (tertian) therapy in *early* cases of paresis is yielding promising results since about 30 to 40 per cent. make clinical recoveries while 20 to 30 per cent. additional show distinct improvement; this is especially true of types of characterized by excitement and expansive ideas as also cases exhibiting dementia and depression. The galloping juvenile and infantile types are not much benefited. Relapses after improvement appear to occur in at least 10 per cent. of cases and these may be safely re-inoculated with malaria. But these results are not any better, and, indeed, not quite as good, as those recently reported in this country by Lorenz, Moore and others, following treatment with tryparsamid. For this reason I doubt if malarial therapy will be adopted in America as enthusiastically as abroad and especially if the results already observed with tryparsamid receive corroboration by further experience.

Mechanism of Malarial Therapy of Paresis.—The mechanism by which malarial and relapsing fever infections produce remissions in paresis has been a subject of considerable speculation. As previously stated, the basis for investigating the effects of malaria by V. Jauregg-Wagner appears to have been the beneficial effects of the fever, which he had produced previously with injections of tuberculin and sodium nucleinate. In this connection it is interesting to note that Weichbrodt and Jahnel[178] have studied the influence of fever on syphilis by exposing infected rabbits to 42–43°C. for varying periods of time and state that a favorable influence was exerted upon the disease. Some have sought an explanation on the basis of meningeal hyperemia and other changes produced by malaria which results in an increased permeability of the tissues for spirocheticides like arsphenamin or neoarsphenamin generally administered after ten or twelve paroxysms; as previously stated histological studies of the brains of paretics treated during life by malarial inoculations, have shown

changes indicating improvement and of special significance, a marked reduction in the numbers of spirochetes in the tissues. The whole subject has been placed by most investigators into the category of non-specific protein therapy, but I believe this only confuses the problem and adds nothing to its solution.

As previously stated, it has been for years a matter of clinical experience that a disease may undergo retrogression when a second infection is superimposed. For example, in 1916, Zupnik, Miller, and Leiner[179] showed that malarial paroxysms in typhoid fever frequently bring about rapid clinical improvement of the latter, and the effect of erysipelas on sarcomata and of pregnancy on tumors are related phenomena. In other words the superimposed or secondary infection appears to stimulate immunological resistance of the tissues to the first infection; in this instance the malaria or relapsing fever parasites appear to greatly stimulate the resistance of the cerebral tissues to *Sp. pallida* which may result in producing a prolonged period of latency of the spirochetes and probably even the destruction of many of them. For this phenomenon the term "*stimulating immunity*" may be employed and this is quite similar to the "*depression immunity*" of Morgenroth, Biberstein and Schmitzer[180] who, in 1920, observed that mice infected with streptococci, develop in four to twenty-four hours a temporary and relative resistance to a second infection of the same or other streptococci in doses that prove fatal for control animals. In other words, the first infection appears to depress the reaction of the tissues to the second, but I prefer to think of the first infection as stimulating the resistance of the tissues as a whole to added infections of the same or other parasite and regard the influence of malaria and relapsing fever upon paresis as an example of *non-specific stimulating immunity*, the superimposed infection stimulating resistance to *Sp. pallida* in a non-specific manner.

Author's Method for the Specific Treatment of Early Asymptomatic Neurosyphilis.—Under this heading I refer to those cases of "spinal fluid syphilis" showing spinal fluid abnormalities without clinical signs or symptoms during the first six months to year or so after contracting the disease. In practice they constitute the approximate 20 to 25 per cent. of cases showing such spinal fluid changes as a slight increase of cells and protein, a weakly positive Wassermann reaction and possibly a colloidal gold curve of the luetic zone type, when examined after the first year of treatment following the chancre.

The treatment of this type of case is that of primary and secondary syphilis described in Chapter LXXII. As a general rule, intraspinal therapy is not required, although Gennerich[181] states that combined intravenous and intraspinal therapy is indicated and advises two intraspinal treatments after the spinal fluid has returned to normal followed by spinal fluid examinations every two or three months during the first six months after cessation of specific medication with subsequent examinations every six months for two or more years thereafter to detect possible relapses. When spinal fluid abnormalities are observed it is not advisable to ignore them or to trust to mercury by ingestion for their removal. More vigorous treatment should be continued and I am now employing sulpharsphenamin by intramuscular injection combined with mercury by inunction, giving a course of six weekly injections of the former in dose of 0.4 to 0.6 gm. with three inunctions of mercurial ointment per week (making 18 in the series). After two months rest this course is repeated or, if the sulpharsphenamin is objected to, eight weekly intravenous injections of neoarsphenamin are given with three weekly inunctions. If the inunctions are objected to, I give two weekly intramuscular injections of bismuth tartrobismuthate in dose of 0.2 gm., the course, under these circumstances, consisting of 8 injections of neoarsphenamin and 16 injections of bismuth, the latter totalling 3.2 gms. After another period of rest of several months, during which mercury by ingestion may be given, the spinal fluid is again examined. If still showing abnormalities, I believe that it is well to consider a series of four to six Swift-Ellis treatments consisting of an injection of either 0.6 gm. arsphenamin or 0.9 gm. neoarsphenamin every two weeks followed by the withdrawal of blood one half hour later and the intraspinal injection of serum next day as described on page 872. Tryparsamid should not be used, because experience has

shown that it is not especially indicated in this kind of case and there is risk of optic involvement.

Author's Method for the Specific Treatment of Late Asymptomatic Neurosyphilis.—This type of case refers to those discovered by spinal fluid examinations two years or more after the chancre stage. As a general rule, the interval is five to ten years or longer. There are no discoverable clinical signs or symptoms and sometimes not the slightest signs of fixation of the pupils. The spinal fluid shows an increase of cells and protein, a positive Wassermann reaction and a luetic zone colloidal gold curve; or the Wassermann reaction may be strongly positive with a paretic zone colloidal gold curve constituting what Moore and others have designated as "asymptomatic paresis" and corresponding to Southard's "paresis sine paresi." Not infrequently, however, the latter show some slight signs of neural involvement although there are no mental symptoms.

The treatment of such cases, and especially the latter, should be sufficiently vigorous and particularly since many are simply in the preparetic or pretabetic stages of neurosyphilis:

1. It is well to commence treatment mildly in order to avoid Herxheimer reactions, if more than a year has elapsed since the last course of treatment. The usual preparatory treatment is given in the way of correction of focal infections, advice regarding general hygiene and particularly of the mouth, etc.; alcohol should be forbidden or used in great moderation. For the first month specific medication may be of daily inunctions of mercury along with iodids by ingestion or weekly epifascial injections of 1 grain of succininmid of mercury or weekly intramuscular injections of bismuth tartrobismuthate in dose of 0.2 gm.

2. This is followed immediately by two months of arsenical treatment of which a choice may be made of the following: Weekly intramuscular injections of sulpharsphenamin in dose of 0.4 gm.; or weekly intravenous injections of tryparsamid in dose of 2.0 gm.; or weekly intravenous injections of neoarsphenamin in dose of 0.6 to 0.9 gm.; or weekly intravenous injections of arsphenamin in dose of 0.4 gm. I have named them in the order of my own preference. At the same time, it is well to administer mercury either in the form of weekly epifascial injections of the succinimid in dose of 1 grain or three weekly inunctions of mercurial ointment.

3. This is followed by a rest of one or two months when the course is repeated.

4. After another one or two months of rest, during which mercury by ingestion may be given, an examination of the spinal fluid is made along with a blood Wassermann reaction. If the reactions are negative, subsequent treatment may be milder in the form of courses of mercurial inunctions combined with iodid by ingestion and finally tapering off with mercury by ingestion.

5. If, however, the serological reactions are still positive it is well to continue with more vigorous combination therapy of mercury by inunction or intramuscular injection with intravenous injections of 3 gm. of tryparsamid every two weeks or weekly intramuscular injections of sulpharsphenamin, giving 6 to 8 injections of either or 3 to 4 alternating injections of each, as a course.

6. After a period of two to three months rest, during which, however, mercury by ingestion may be given, the blood and spinal fluid tests should be repeated. If negative reactions occur, the balance of treatment may be of mercury and iodids, gradually tapering off. If positive reactions are observed, however, more vigorous treatment is required in the form of com-

bined intravenous and intraspinal treatments by the Swift-Ellis or my own method at the rate of one every two weeks for a series of at least three.

7. Some of these cases are "Wassermann-fast" and demand periodical courses of treatment for several years as described in Chapter LXXV. Each case must be judged by its own special indications and the treatment advised is only in the nature of suggestions subject to modification. Even if negative blood and spinal fluid reactions are observed, it is well for the patient to continue under observation for several years with periodical mild courses of treatment to the extent at least of two months' courses of mercury and iodid by ingestion followed by periods of rest, after Fournier's chronic intermittent treatment (see page 1050).

Author's Method for the Specific Treatment of Meningovascular Neurosyphilis (Cerebrospinal Syphilis).—Under this heading I include cases sometimes possible of diagnosis on the basis of headache, dizziness and other signs present in paresis but without mental changes and presenting spinal fluid changes of high cell counts (due to meningitis), moderate protein, moderately positive Wassermann reactions and colloidal gold curves of the luetic rather than the paretic zone type. Many borderline cases occur however, in which the paretic gold curve occurs and sharp differentiation from incipient paresis cannot be made. As a general rule, there are also clinical evidences of myocarditis and aortitis.

In this type of case I believe that sulpharsphenamin and tryparsamid are to be preferred to arsphenamin and neoarsphenamin because less likely to produce vascular injuries; also bismuth is to be preferred to mercury or at least should be given interchangeably with mercury and the iodids are not to be omitted. Bismuth is especially indicated.

1. After the usual preparatory and hygienic treatment, specific medication should begin mildly in order to avoid Herxheimer reactions. My practice is to give weekly intramuscular injections of potassium tartrobismuthate in dose of 0.2 gm. for eight injections along with daily doses of iodids by ingestion.

2. This is followed by a course of eight weekly epifascial injections of sulpharsphenamin in dose of 0.4 gm. or weekly intravenous injections of tryparsamid in dose of 2.0 gms. If these are not given, neoarsphenamin may be injected intravenously in dose of 0.6 to 0.9 gm. once a week for ten weeks.

3. This is followed immediately by a course of daily inunctions of mercuro with iodid by ingestion for six weeks, the urine being examined weekly.

4. After a rest of two months the blood and spinal fluid are examined. If negative reactions are observed it is well to give an additional course of injections of sulpharsphenamin or neoarsphenamin or at least continue specific treatment with courses of mercury by inunction or injection or bismuth by injection, finally tapering off with mercury and iodids by ingestion.

5. If positive spinal fluid reactions are observed, even though the blood Wassermann reactions are negative, more energetic treatment is required and especially a combination of systemic and intraspinal therapy by the Swift-Ellis or some similar method. Patients presenting strongly positive spinal fluid Wassermann reactions with paretic curve colloidal gold reactions, are especially in need of vigorous treatment and careful clinical observation. My practice is to give a series of four or more Swift-Ellis treatments, substituting 0.9 gm. neoarsphenamin for 0.6 gm. arsphenamin, at intervals of two weeks. These are followed by a course of mercury by inunction and further treatment as indicated.

Author's Method for the Specific Treatment of Vascular Neurosyphilis.—Under this heading I refer to hemiplegia and thromboses due to syphilitic

infection of the blood vessels of the central nervous system. Practically all of these cases likewise show clinical or roentgenologic evidences of myocarditis and aortitis.

Treatment is indicated for the purpose of preventing, if possible, further vascular injuries and accidents as stated by Milian,[182] Nicand[183] and others, since great improvement may result. But great care is required in the selection and administration of remedies. As far as possible, intravenous injections should be avoided and particularly if any undue excitement attends the injections. Intramuscular injections of sulpharsphenamin, with mercury by inunction or injection, and large doses of iodids by ingestion, constitute the medicaments of choice. If sulpharsphenamin cannot be given, one may try small doses of neoarsphenamin (0.1 to 0.3 gm.) by intravenous injection, as described in Chapter LXXV for the treatment of chronic cardiovascular syphilis. Arsphenamin should not be used; tryparsamid is not especially indicated. In grave cases of cardiovascular syphilis when intravenous injections may be contraindicated the specific treatment of neurosyphilis may be conducted by the Ogilvie method of intraspinal medication, but in hemiplegia it is imperative to conduct spinal punctures and subarachanoid injections with more than usual care and, I believe, this should not be resorted to at all except under well controlled conditions providing for measurement of spinal fluid pressure and the prevention of undue excitement, increase of blood pressure and too sudden reduction of spinal fluid pressure.

Author's Method for the Specific Treatment of Tabes Dorsalis.—Many different methods or plans have been proposed for the treatment of tabes dorsalis. In early cases specific medication usually yields particularly gratifying results in the relief of pain and other symptoms but in all cases the chief purpose is to stop further progression of the disease with the realization that any nerve structures already destroyed cannot be restored, although the relief of pressure from edema and exudates upon nerve cells may result in a partial restoration of function.

1. It is particularly advisable to initate treatment by a thorough overhauling of the patient with special reference to the correction of focal infection, dietetic errors, constipation, alcoholic and other excesses, etc. Massage and educational muscular exercises may be indicated and especially in well-developed cases.

2. The first month of specific medication may be a thorough course of mercury by inunction with iodids by ingestion according to the Aachen or Hot Springs methods, as described in Chapter LXIII. It is imperative not to produce an exacerbation of the syphilitic processes (Herxheimer reactions) which may increase bladder, rectal and other disturbances and prove very difficult to check or control.

3. This is followed by a series of alternate intravenous injections of tryparsamid, in dose of 2 to 3 gm., and epifascial injections of sulpharsphenamin in dose of 0.4 gm., giving one injection per week for a course of twelve weeks (6 injections of each). If tryparsamid is not given, 8 to 10 injections of sulpharsphenamin may be administered; or 12 weekly intravenous injections of neoarsphenamin beginning with 0.45 gm. and increasing, or 6 weekly intravenous injections of arsphenamin beginning with 0.2 gm.

4. Immediately after this course or after a rest of one month, mercury and iodids should be given again for six to eight weeks, the former by inunction if possible. If inunctions cannot be given, potassium tartrobismuthate in dose of 0.2 gm. or the succinimid of mercury in dose of 1 grain may be injected intramuscularly once a week for eight to ten weeks.

5. After a rest of two or three months a blood Wassermann test and spinal fluid examination should be made. If satisfactory progress has not be obtained, intraspinal medication should be considered by the Swift-Ellis, Olgivie or some similar method. My preference is for the straight Swift-Ellis method every two or three weeks for a series of four or more, because this method produces the least cord and meningeal irritation and it is imperative to reduce these reactions to a minimum. The relief afforded by Swift-Ellis treatments is frequently so striking, and especially in early cases, that it is unwise to delay too long employing them in treatment. I believe they should be used whenever fairly energetic systemic treatment as outlined above, proves insufficient for checking the disease.

6. Subsequent treatment is guided by individual circumstances. As long as the blood and spinal fluid reactions are unchanged, specific medication should be continued, the kind and degree depending upon the tolerance and general condition of the patient. Among the arsenicals it would appear that preference is to be given to sulpharsphenamin, tryparsamid and arsphenamin in the order mentioned. Mercury by inunction and large doses of iodid by ingestion should be included. I am especially impressed with the value of combination therapy in these cases, giving as a course for eight weeks, 3 inunctions of mercury and 1 epifascial injection of sulpharsphenamin or 1 intravenous injection of tryparsamid per week along with daily doses of 40 to 60 grains of potassium or sodium iodid by ingestion. The number of courses given depends upon circumstances; at least two or three months of rest without treatment or with mercury by ingestion should be allowed.

7. In cases developing negative blood Wassermann and spinal fluid reactions, the degree of specific medication may be reduced, because a clearing up of the serology is almost always a safe indication of cessation of progress of the disease. In such instances I usually prescribe a course of eight weeks of mercurial inunctions with iodid by ingestion about three times a year for the following two years and then once or twice a year thereafter. If inunctions cannot be given, weekly intramuscular injections of either the succinimid of mercury or potassium tartrobismuthate are substituted. During the rest periods protiodide of mercury is generally given for a month or two at a time.

8. Sometimes in the course of specific treatment I administer an occasional intramuscular injection of one of the commercial milk preparations for the benefits accruing from non-specific protein reactions, especially in advanced cases, obviously under weight and of poor nutrition.

Author's Method for the Specific Treatment of Paresis and Taboparesis. In the management of active progressive cases of paresis and taboparesis, energetic specific medication is required and the results recently observed in cases treated with tryparsamid offers somewhat more hope for success than formerly. But even if tryparsamid is not employed, it should not be forgotten that energetic systemic treatment with arsphenamin and sulpharsphenamin combined with subarachanoid medication offers a good deal and especially in the treatment of early cases.

1. As in the treatment of all forms of chronic syphilis, it is required to pay particular attention to the removal of foci of infection and the correction of hygienic errors. Alcohol should be forbidden, as emphasized by Kelly,[184] who states that it may add to the gliosis of the brain in paresis. Physical and mental strain should be reduced to a minimum, although massage and exercise should not be overlooked. Constipation should be corrected.

2. Specific treatment may be initiated by giving six weeks of daily inunctions of mercury along with weekly intramuscular injections of potassium tartro-bismuthate in dose of 0.2 gm. and 40 to 60 grains of potassium

iodid daily by ingestion. If, however, the disease is rapidly progressing this initiatory treatment may be shortened with the prompt institution of arsenical treatment.

3. It would appear advisable to give 1.5 grams of tryparsamid intravenously once every week or 2 grams every two weeks for eight to ten injections. Otherwise one may give 0.4 to 0.6 gm. of arsphenamin or 0.9 gm. of neoarsphenamin intravenously or 0.4 gm. of sulpharsphenamin epifascially, once a week for six to ten weeks. A good plan is to give tryparsamid alternately with sulpharsphenamin. At the same time mercurial inunctions are given three or four times a week with 40 to 60 grains of iodid daily by ingestion or if the inunctions cannot be given, the succinimid of mercury may be injected intramuscularly once a week in dose of 1 to 1½ grains.

4. This is followed immediately or after a rest of one or two months if the disease has been checked, by a course of combined intravenous and subarachanoid treatments. My practice has been to use the Swift-Ellis method with reinforcement of the serum with 0.0005 gm. of arsphenamin or 0.001 gm. of neoarsphenamin. These are given every two or three weeks, the number depending upon the tolerance and reactions of individual cases. I have not used intraventricular and intracisternal injections, but believe that these routes, and especially the former, are deserving of more attention than they have hitherto received and particularly in the institutional treatment of severe progressing cases. During this period it may be advisable to give alternate intramuscular injections of either the salicylate of mercury or potassium tartrobismuthate in addition to the arsenicals.

5. It would appear that non-specific protein therapy may be worth while during the course of specific treatments, in the form of an occasional intramuscular injection of 0.5 gm. of sodium nucleinate or 5 c.c. of sterile milk or one of the commercial milk preparations. If tryparsamid is employed these may not be required, if the nutritional effects of this drug are produced.

6. The purpose of treatment is to produce clinical remissions. Even if serological remissions occur, treatment should not be interrupted unless there is a corresponding degree of clinical improvement. As long as energetic treatment is indicated, the courses described in paragraphs 3 and 4 may be repeated with suitable rest periods.

7. But it is not advisable to stop treatment during remissions, even in those remissions designated as "recoveries." This state is almost too good to be truly lasting and should be maintained by courses of mercury by inunction or intramuscular injection or occasional short courses of tryparsamid or sulpharsphenamin. Mental stress and hard work should be forbidden. Even under the best of circumstances it would appear that most cases in remission are a little "off" or "queer" and it is a mistake to entrust them with responsibilities until one is sure of their capabilities.

8. Malarial or relapsing fever therapy may be used and especially the former, but if the results reported with tryparsamid are substantiated by further experience, I see no particular use of resorting to malarial therapy in ordinary private practice, since the results produced by tryparsamid and sulpharsphenamin with or without subarachanoid medication, appear to equal or even exceed those reported from Europe with malarial therapy.

Prognosis in Neurosyphilis in Relation to Specific Treatment.—The prognosis of syphilis in general in relation of specific therapy is discussed in Chapter LXXIX but it may not be amiss at this place to briefly refer to the subject in relation to the treatment of neurosyphilis.

All writers on the subject have emphasized the importance of early and thorough treatment of syphilis as the best means for preventing neurosyphilis

and likewise as the best means for curing infection of these tissues. Neurosyphilis may be checked, but is seldom cured by haphazard and incomplete treatment; in such, relapses are frequent and usually due to residual infections. As so well stated by Collins,[185] the earlier treatment is instituted, the greater is the victim's chances of complete functional recovery and without those "scars of his mind and his emotions which permanently cripple him to some degree." For this reason the necessity for the early diagnosis and thorough treatment of neurosyphilis imposes a heavy responsibility upon all physicians undertaking the treatment of syphilis.

Neurosyphilis demands thorough treatment for a favorable prognosis; Harrison[186] states that he does not undertake its treatment at all and especially tabes, unless the patient is willing to persevere until he has received a substantial amount of the organic arsenicals; in his opinion it is better to withhold these unless thorough treatment can be given.

Best results are secured in the meningeal and vascular types including early cases of tabes, before ascending degeneration of the posterior columns of the spinal cord takes place. Advanced degenerations are of course irremediable; as stated by Harrison, "we may put out the fire but we can do nothing toward rebuilding the house and nature can do little." But pains and crises in tabes due to associated neuritis can be relieved. Furthermore, as stated by Fordyce, tabes progresses because the cause persists and as fresh segments of the cord are involved; this can be prevented in many cases by appropriate specific medication. It is true that spontaneous arrest of tabes and even of paresis may occur under ordinary treatment or with no treatment at all, as a result of the natural defensive agencies of the body, but, as stated by Fordyce, these should only encourage us the more in therapeutic endeavors. Solomon[187] believes that the majority of cases of syphilis of the nervous system, whether the so-called cerebrospinal syphilis, tabes dorsalis, general paralysis or other forms, are entitled to treatment, and if this is done thoroughly, intensively and systematically the results will be gratifying.

Thompson,[188] in a recent review of 53 cases of neurosyphilis treated by vaious methods concludes that intraspinal therapy is of value in neurosyphilis, that it should be given in connection with arsphenamin and mercury and that it is advisable at periodic intervals as long as the patient remains clinically or serologically positive.

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CHAPTER LXXVII

SPECIFIC TREATMENT OF CONJUGAL AND CONGENITAL SYPHILIS

Some of the most difficult and perplexing questions in relation to the specific treatment of syphilis arise in connection with the subject of marriage and parenthood. These do not refer to those cases of syphilis in either sex contracted and thoroughly treated before marriage, but rather to those in whom evidences of syphilis are discovered after marriage and especially after children have been born. Can the father have syphilis without infecting either mother or child? Can the mother have syphilis without infecting the father or the child? These are matters that concern the question of specific treatment of the marital partners in such unions and it is in this sense that I am employing the term "conjugal syphilis" We are fairly certain of what should constitute the criteria of cure before marriage and parenthood may be safely sanctioned, but numerous cases arise in which a father is found to present evidences of syphilis after marriage of several years which immediately raises the question: Are the mother and children infected and thereby in need of specific medication, even though they do not show any definite clinical

or serological evidences of the disease? What is the proper procedure when a mother is found syphilitic under similar circumstances? Should the husband be treated? Should the children? Are we in position at present to lay down broad and uniform rules for the management of such cases? What are the criteria by which decisions may be reached, and no one experienced in the treatment of syphilis will deny the tremendous practical importance of these questions, involving as they may, the domestic peace and happiness as well as the health and even the lives of innocent and unsuspecting individuals. It is not so much a question of *how* such cases should be managed from the standpoint of specific therapy, because this is along the lines of the treatment of syphilis in general, but rather the question of diagnosis and the criteria by which decisions are reached regarding whether or not the spouse and children require antisyphilitic medication.

Great advances have been made in our exact knowledge of conjugal and congenital syphilis during the past twenty years by means of the Wassermann test and especially during the past ten years by careful and extensive clinical, sociological and serological studies employing improved methods, but there are still many and wide gaps in our information on these subjects. Percentages expressing the incidence of infection under this or that condition aid us in arriving at decisions, but in many cases it is after all a matter of expressing opinions with a full realization that only the future welfare of the individuals concerned can tell us if we are right or wrong.

In this chapter I shall endeavor to present my opinions of the subjects concerned and the reasons upon which they are based, along with methods for the specific treatment of various types of congenital syphilis; the subject of conjugal syphilis will be discussed only from the standpoint of whether or not specific medication is indicated, because if this is decided upon, the methods advised have been already presented in preceding chapters.

Terminology.—But before entering upon a discussion of these subjects it may be well to define the meaning of the terms conjugal and congenital syphilis as employed in this chapter. *Conjugal syphilis* refers to the infection of the spouse (man or woman) by reason of the marital relation and will be discussed from the standpoints of diagnosis and specific therapy. *Congenital syphilis* refers to the infection of a fetus in the uterus with *Sp. pallida*. It does not include, therefore, those exceptional cases of syphilis of children contracted during birth or shortly thereafter by contact with chancres, mucous patches or other open lesions of the mother or wet nurse; these constitute examples of *infantile syphilis*. The term *prenatal syphilis* may be used as a synonym for congenital syphilis, but the terms *hereditary syphilis* and *heredo-syphilis*, so frequently employed by French writers, are misnomers, because an actual parasite and disease are transmitted rather than a physical or mental trait or characteristic of the parents. *Conceptional syphilis* is applied to those cases of congenital syphilis in which it is believed that *Sp. pallida* infects the ovum at the time of conception: by paternal transmission, if the parasite is contained in the semen, or by maternal transmission, if the parasite is from the blood or tissues of the woman.

Syphilis and Marriage in Relation to Specific Therapy.—Owing to the frequency with which syphilis is transmitted to the spouse and offspring, the subject of syphilis and marriage has very properly attracted a great deal of attention and especially in relation to public health. Many statistical studies have been published which need not here be reviewed, as our present interests concern the matter of how much specific treatment should be given either the man or woman before marriage may be sanctioned, in order to be fairly sure that the spouse and offspring will not be infected. In a way, the subject resolves itself into a discussion of the criteria of cure because the only safe time to marry is when there are good reasons for believing that the individual has been cured. Furthermore, our standards and criteria are more strict than laid down by Fournier and other older syphilologists, because they had only clinical signs for their guidance, whereas we have the Wasser-

mann test in addition, which has added very materially to our knowledge of conjugal and congenital syphilis during the past fifteen years.

Since physicians are very frequently asked questions bearing upon marriages involving syphilitic individuals, I am stating here the rules by which my opinions are guided, these being in conformity with the practice of many leading and experienced syphilologists:

1. Patients seen and placed upon intensive treatment during the chancre stage and before the Wassermann reaction has become positive may be allowed to marry after at least one year of vigorous treatment followed by a second year of observation and milder treatment as described in Chapter LXXII, provided there have been at no time secondary manifestations or serological evidences of infection in the blood and spinal fluid.

2. Patients seen and placed upon intensive treatment during the late primary stage or in the secondary stage after the Wassermann reaction has become positive may be allowed to marry after two years of vigorous treatment followed by a third year of milder treatment and observation, during which there have been no clinical manifestations of the disease, repeatedly negative blood Wassermann reactions and at least one complete examination of the spinal fluid with negative results.

3. Patients seen in the latent and tertiary stages of syphilis with positive Wassermann reactions, may be allowed to marry after thorough courses of treatment over a period of at least two years followed by a third year of milder treatment and observation as described in Chapter LXXV, during which there should be absolutely no clinical evidences of syphilis, repeatedly negative blood Wassermann reactions and at least one complete examination of the spinal fluid with negative results. A provocative Wassermann test is advisable as described on page 519.

4. A *man* with a persistently positive blood Wassermann reaction may be allowed to marry if there has been at least three years of vigorous treatment and if the spinal fluid shows no abnormalities; in the majority of such instances both the spouse and children escape infection, *but it cannot be stated that the union is absolutely free of the danger of transmission of infection* and many physicians, including myself, naturally hestitate to sanction marriage under these conditions. A *woman* under these conditions, however, should not marry at all because she incurs a much greater risk of transmitting the disease to offspring; there is likewise a slight risk of transmission to the spouse.

5. A *man* or a *woman* with a persistently positive blood Wassermann reaction and the spinal fluid changes of neurosyphilis should not marry, regardless of the amount of specific treatment received, because under these conditions, there is more risk of transmission to both spouse and children.

The above mentioned conditions are definite and probably most physicians will agree in whole or most part with the conditions laid down in relation to marriage, but there is a larger group of more puzzling cases in which an opinion must be very guarded because of the impossibility in some cases of detecting latent syphilis by the most thorough physical, serological and roentgenological examinations. Such cases are somewhat as follows:

6. If the patient is certain of having contracted syphilis several years previously, followed by a period of specific treatment, I believe that marriage may be sanctioned if a thorough physical examination reveals no evidences of the disease and the blood and spinal fluid reactions are negative. I refuse an opinion unless a spinal fluid examination is permitted and, I believe, that a provocative Wassermann test is advisable. If the latter is conducted, the spinal fluid examination should be made when the provocative blood tests are conducted, in order to obtain the value of provocative spinal fluid reactions, if such occur. If in spite of negative and favorable results of these kinds the patient and the physician wish to be on surer ground, a year or two of treatment may be given during which repeated physical and serological examinations may be made. In this case the year of specific treatment replaces the provocative injections of arsphenamin or neoarsphenamin and it may be fashioned along the lines described in Chapter LXXV for the treatment of asymptomatic late syphilis.

7. If the patient, an old syphilitic, is unable to give a definite and clear account of his infection and of his previous treatment, greater caution is required. Likewise in cases of either sex with indefinite but suggestive venereal histories, a woman with a history of miscarriages, etc., a thorough physical examination should be made along with blood Wassermann and complete spinal fluid examinations. A provocative Wassermann test is advisable and I always advise one probationery year during which at least two physical and four blood Wassermann tests are made; it is not usually necessary or advisable however, to repeat the spinal fluid examinations. If these examinations fail to disclose evidences of the disease, marriage may be sanctioned but it is necessary to employ a sensitive complement-fixation test because of the danger of falsely negative reactions in the presence of extremely latent syphilis.

Conjugal Syphilis in Relation to Antisyphilitic Treatment of the Spouse.—Under this heading I wish to discuss with a little more detail the question of transmission of syphilis to the partner of a sexual union in relation to the matter of specific treatment, before taking up the subject in relation to the offspring. Whenever syphilis is discovered in a husband or wife, the question of the management of the spouse arises and frequently presents difficult problems and situations.

It scarcely requires stating that a syphilitic of either sex, with open lesions and especially chancres and mucous patches, runs grave risks of infecting the sexual partner and especially by repeated contacts. In addition there are risks of extra genital infection and marriage at least, can never be sanctioned under such circumstances.

But in the presence of clinically latent syphilis without demonstrable open lesions, what are the chances of infection of the spouse? The subject must be discussed separately according to the sex of the syphilitic partner.

A syphilitic husband under these conditions is far more likely to infect his wife than is a syphilitic wife likely to infect her husband. She runs the risk of infection by three ways, namely, contact with an open lesion of the husband which may develop at any time; contact with his semen carrying *Sp. pallida*, and thirdly, the possibility of infection from a syphilized fetus, if it is granted that paternal conceptional syphilis may occur. The husband, however, is liable to infection from a syphilitic wife only in case he comes in contact with open lesions, if such develop in the course of years.

Infection of a wife by contact with the open lesions of a husband requires no discussion; if one can be certain that the disease has been latent through the period of marital relationship, infection of the wife by this route may be excluded. But I believe she may be infected by the semen direct without involving the question of pregnancy and especially if her husband's testicles are infected with *Sp. pallida*, as is now regarded of frequent occurrence even in the absence of clinical signs of infection. In such cases the disease may run a very mild course without its presence being suspected, the wife being entirely ignorant of infection (*syphilis ignota*), because the spirochetes are of greatly reduced virulence.

But is it possible for a woman to carry a syphilitic fetus and yet escape the disease? Some physicians believe that *Sp. pallida* or rather a smaller spore-like form of the parasite may infect the ovum direct (*paternal conceptional syphilis);* but may the mother escape through the destruction of spirochetes by placental ferments as stated by Routh[1] or some other means? Jeans[2] has found that 85 per cent. of mothers of syphilitic infants gave positive Wassermann reactions. But what is the status of those who do not and who present no clinical evidences of the disease? For my own part I believe that we should regard all mothers of syphilitic children born at or near term, as syphilitic and place them on antisyphilitic treatment. It may be that a woman aborting a syphilitic fetus, before the third month, may escape infection but I doubt this and feel absolutely sure that all women with a history of two or more syphilitic pregnancies should be placed on antisyphilitic treatment. Indeed, an increasing number of syphilologists believe that paternal infection of the ovum does not occur and that a syphilitic fetus always indicates that the mother is syphilitic and that she was infected before conception occurred. In other words, according to this view, a syphilitic fetus always indicates syphilitic infection of the mother and I subscribe to this opinion at least to the extent of always advising antisyphilitic treatment of the mother even though her blood Wassermann test may yield a negative reaction.

Indeed, I know of no other type of syphilis more likely to yield *falsely negative* Wassermann reactions unless it be latent congenital syphilis of children.

However, it must be granted that a woman married to a man infected with syphilis five or more years previously, may escape seminal and fetal infection if her husband's disease has been latent. But our opinion in each individual case must be based only upon thorough physical and serological examinations because if the wife is syphilitic, the disease is apt to be very latent and defy detection even by thorough and sensitive tests. However, we must have some criteria for judging whether or not the wife of a syphilitic man requires antisyphilitic treatment and I believe that treatment may be *omitted* under the following conditions: (*a*) When there are no manifest or suspicious clinical evidences of syphilis; (*b*) when repeated Wassermann tests by a sensitive and acceptable method yield negative reactions; (*c*) when there is no history of spontaneous abortions or miscarriages; (*d*) when the child or children show no clinical or serological evidences of syphilis. To these may be added negative provocative blood Wassermann and spinal fluid reactions, although these cannot usually be applied when conditions demand or warrant the secret investigations of spouse and offspring.

But if these are acceptable as criteria for the omission of specific medication for the wife of a syphilitic man, what are the indications for the institution of treatment? I believe these may be stated as follows: (*a*) The presence of even suspicious clinical manifestations even though Wassermann reactions are negative; (*b*) the occurrence of a positive Wassermann reaction, even though there are no clinical manifestations; (*c*) the birth of a syphilitic fetus or child even though there are neither clinical manifestations nor positive Wassermann reactions in the mother. If the blood Wassermann reactions are negative but the spinal fluid shows abnormalities, treatment should be instituted.

As a general rule, most reliance is placed upon the results of Wassermann tests and especially since these are frequently the best examinations possible when conditions demand an investigation without disclosing its purpose. As repeatedly stated it is imperative that these tests shall be done with a method as sensitive as consistent with specificity because even under these circumstances we are always apt to secure falsely negative reactions. After a woman has lived with a syphilitic man for several years we can never be absolutely sure of her having escaped infection; for example, as shown by Veeder[3] at least 86 per cent. of mothers of syphilitic children yield positive Wassermann reactions indicating syphilitic infection even though there are no demonstrable clinical evidences and the women are ignorant of being infected (*syphilis ignota*). At best we may say that in the absence of all manifest or suspicious clinical and serological evidences of infection, that antisyphilitic treatment need not be given but that there should be subsequent and periodic clinical and serological examinations over a period of several years. As a general rule these may be managed even when it is necessary to proceed secretly in order to preserve domestic peace and happiness. But no physician should be a party to any agreement by which the spouse is neglected and left to her fate; with tact and good judgement the physician may readily do his duty even in difficult situations when suspicion has greatly sharpened the intellect of the spouse.

The reversal of these conditions may present just as puzzling a situation, that is, when syphilis is brought into a marital relationship by the woman. If she presents early open lesions it is almost sure that the husband will become infected with the production of a chancre and the usual clinical course of syphilis. But in the absence of these, what are the chances of

infection of the husband when the disease in the wife is chronic and latent with no demonstrable open lesions? The possibilities of his contracting the disease are very much less than when the conditions are reversed and the syphilitic partner is the male. But yet there is danger of infection and especially after marriage of several years. Evidently small open lesions may develop about the woman which do not attract attention and curiously enough, the man may not notice a chancre or other manifestations of sufficient degree to attract attention, the presence of the disease being unsuspected because he is ignorant of the possibilities of infection. Numerous instances are on record of neurosyphilis in the husband contracted from marriage with a neurosyphilitic woman although in most cases the reverse pertains. Evidently the spirochetes, derived from small undetected lesions about the woman, are of greatly reduced virulence and produce undetected lesions at the point of entry. Therefore, if syphilis has been introduced into a marriage by the wife, the husband should be examined for evidences of infection and when this must be secretly done, I know of no more difficult situation. As a general rule, it is possible to arrange for a physical examination and a blood Wassermann test at least and if the latter is positive by a sensitive and acceptable method, it may be assumed that the husband is infected and in need of specific treatment. If these examinations are negative a provocative Wassermann test and examination of the spinal fluid should be done if possible; if these are negative we may at least state that there are no indications for antisyphilitic treatment although we may not be absolutely sure that latent syphilitic infection is absent.

When syphilis is discovered in either spouse under such conditions, it is almost impossible to give thorough treatment without disclosing its purpose; at least, in my experience, it is difficult to secure proper cooperation until the patient understands the purpose of treatment. I have in many cases, however, given courses of neoarsphenamin by intravenous injection on the assumption that these were required for the correction of anemic states and have succeeded in administering mercury by inunction in the form of 85-per cent. calomel ointment under the disguise of massage. Mercury and iodid at least may be easily given by ingestion over long periods of time after Fournier's chronic intermittent method described on page 1050. But if possible the plan of therapeutic attack should be along the same lines as advised in Chapter LXXV for the treatment of chronic syphilis according to the condition of the spouse, although modifications are usually demanded by the individual conditions and circumstances of each patient.

Conjugal Syphilis in Relation to Antisyphilitic Treatment of Children.—Having thus considered the matter of antisyphilitic treatment of the spouse involved in a marriage with a syphilitic, we may now take up the subject of treatment of the children of such a union. Of course, if the child shows clinical or serological evidences of syphilis, treatment is to be given regardless of which marital partner is responsible for the disease. But not infrequently the matter of specific medication is difficult to decide when the offspring does not show clear-cut evidences of infection. Here again our decision is greatly influenced by whether it is the mother or father who is syphilitic.

Can a syphilitic father beget a non-syphilitic child? During the early acute stages of the disease the answer is "no" in almost all cases. But in the chronic latent stages it would appear that this is possible and indeed, of frequent occurrence *if the mother has escaped infection.* Under conditions of extreme latency, his semen may not be infectious and both the mother and child may escape infection, insofar, at least, as may be determined by thorough physical and serological examinations. But even though the syphilitic

father has apparently not infected the mother and even though the children are borne without the usual manifestations of syphilis great care and skill are required in the physical examination of the offspring for unusual evidences of syphilis. As pointed out by a commission of French syphilologists composed of Jueyrat, Hudelo, Spillmann, Gastrou and Simon,[4] it is a well-recognized fact that an old syphilitic without infecting his wife, may have offsprings with various dystrophies, although the children may not have active syphilis. It is suggested that these dystrophies may be due to a toxic action on the spermatozoa by the spirochetes in the father.

If the mother is syphilitic either because of infection before or after marriage, it is likely that the child or children are infected. Cooke and Jeans[5] found evidences of syphilis in at least 70 per cent. of the children of Wassermann-positive mothers. Evidences of placental syphilis were observed by Jeans and Cooke[6] in only 27 per cent. of cases, all of these children showing clinical evidences of syphilis, but it is now known that a child may be borne with syphilis without an examination of the placenta disclosing the presence of *Sp. pallida* or histological evidences of infection.

What should influence our decision on the question of antisyphilitic treatment of the offspring if the father is found syphilitic and the mother apparently free? This involves the question of whether it is possible for the father to infect the ovum direct and bring forth a syphilitic child without infection of the mother. Golay[7] and others believe this possible; Moore[8] and others are opposed to the theory. The question cannot be settled in a definite manner and while it may be granted that a small form of *Sp. pallida* may enter the ovum along with a spermatozoön, I cannot conceive how conception can be followed by the development of a fetus unless the parasites are of an extremely reduced virulence, which is highly improbable. In such cases I believe that treatment may be safely omitted if the offspring are in good general health and nutrition, free of dystrophies and if they yield a *series* of negative Wassermann reactions by a sensitive method over a period of several years. I do not believe that a decision can be made on the basis of a single examination; observation and repeated clinical and serological examinations are necessary for reaching a final decision. If, however, the child is subnormal mentally or physically, persistently undernourished and of lowered resistance to bacterial infections, persistently anemic, etc., I believe that we should aim to be on the safe side and advise antisyphilitic treatment even though the Wassermann tests yield negative reactions. Furthermore, it is advisable to examine the spinal fluid since the frequency of neural infections in children with spinal fluid changes has been shown by Findaly,[9] Lavergne,[10] Leitch,[11] Kingery,[12] Tezner,[13] Hunt,[14] Ong and Selinger,[15] Fordyce and Rosen[16] and others; the importance of these fluid examinations in congenital syphilis has not been appreciated as much as the results of these investigations indicate and especially when the blood tests yield negative reactions. Just as we would hesitate to express an opinion upon the absence of syphilis in an adult on the basis of negative blood Wassermann tests, so should we hesitate in cases of late congenital syphilis.

If the mother is syphilitic and the father apparently free of infection or if both mother and father are syphilitic, we should be extra cautious in expressing our opinion on the absence of syphilis because the offspring are so much more likely to be syphilitic. Indeed, I believe they should be regarded as syphilitic and treated accordingly until proven otherwise, although I admit that, according to our best methods of detecting syphilis at present, it is to be stated that a mother with latent syphilis may bring forth a non-syphilitic child. Sylvester[17] believes that every child of a syphilitic mother should

receive some treatment after birth irrespective of negative Wassermann reactions and the absence of symptoms; Almkvist,[18] Fordyce and Rosen,[19] however, believe this practice is an unnecessary hardship and that in the absence of clinical and serological evidences of infection in the offspring of Wassermann positive mothers, treatment may be omitted while the child is carefully observed over a period of years. Such children require extraordinary physical and roentgenological examinations for epiphysitis along with Wassermann tests by a most sensitive method and especially if they present no manifest clinical indications of infection. It should never be forgotten that a negative Wassermann reaction may be followed later by positive reactions and especially in latent infections; for this reason physicians should refuse to express a negative opinion on the results of a single examination or test. We may state that in the presence of negative findings, antisyphilitic treatment is not indicated but be careful to add that this cannot be accepted as an opinion that syphilis does not exist; in other words, such children should be kept under observation with occasional blood tests. On the other hand, a blood test of a child made during the first two weeks after birth may yield a positive reaction to be followed later by a negative reaction. What does this signify? Jeans and Cooke, Fordyce and Rosen and others believe that it may be possible for a child to be born free of syphilis but carrying in its blood for a few weeks the Wassermann antibody derived from a syphilitic mother. I do not have evidence to positively refute this assumption; nor do I deny the possibility of its occurrence, but I believe it is a risky assumption and that the phenomenon may be due to a development of latency of the infection; in short, I believe and practice in the institution of antisyphilitic treatment for infants falling into this category.

May a syphilitic mother beget a non-syphilitic child? If her infection is light and latent the question must be answered "yes" in those cases in which there are absolutely no clinical or serological evidences of syphilis over a period of years. Apparently in such instances the child has escaped because the lesions of the mother did not involve the organs and tissues of generation and because periods of spirochetemia did not occur during the period of pregnancy. But as stated above physicians should exercise great caution in the expression of an opinion on the absence of syphilis in a child where the mother is known to have the disease, unless she became infected after the eighth month of pregnancy, in which case, the child may be borne before infection occurs.

Types of Congenital Syphilis in Relation to Specific Treatment.—It is obvious that the kind and amount of specific medication required in congenital syphilis will vary according to the severity and extent of the disease. That the infection itself is subject to wide variations is well known, since it may range all the way from an infant overwhelmed with *Sp. pallida* at birth and succumbing within a few days, to one born without clinical manifestations and showing no evidences for several years and indeed, in not a few cases, until adolescent and adult ages are reached. The only explanation that may be offered for the wide clinical variations refers to the matter of virulence of the infecting organisms on one hand and immunological resistance of child and mother on the other. If the ovum is infected with *Sp. pallida* at the time of conception it is difficult to understand how it can survive and develop; for my part I cannot accept this at all unless the parasites are of greatly reduced virulence. At all events, if conceptional syphilis does occur it is reasonable to expect intrauterine death of the fetus in most cases with a wide distribution of the parasites in the fetus associated with faults in development of the organs, in the cases of survival.

Congenital syphilis due to acute early syphilis of the parents frequently ends in intrauterine death; if the fetus is born viable there are usually well marked clinical manifestations and a poor prognosis. Congenital syphilis due to late syphilis of the parents however, is generally a much milder infection and with a much better prognosis, because the spirochetes are of reduced virulence, on the one hand, with the possibility of a transmission of immunity principles from mother to child, on the other; however, there is so little evidence in support of the latter hypothesis, that most reliance must be placed upon the question of the virulence of *Sp. pallida* as determining the fate of the child. It is sometimes stated that the well known fact that a syphilitic mother may finally beget an apparently non-syphilitic child after preceding syphilitic children, is due to the acquisition of immunity in the mother transmitted to the child; undoubtedly immunity plays an important rôle, but in my opinion it acts by reducing the virulence of the spirochetes in the tissues of the mother, rather than by placental transmission of antibodies.

Clinically several types of congenital syphilis may be therefore encountered, largely determined according to the virulence or latency of the infection in the parents:

A. *Acute symptomatic congenital syphilis* presenting at birth various skin and mucous membrane lesions, snuffles, visceral involvements, poor physical condition and a positive Wassermann reaction. Many of these infants die in a few days and faulty development, poor musculature and reduced resistance greatly complicate specific therapy. Prompt intramuscular treatment however, frequently works wonders with a rapid transformation of the infant's condition. This type of case, however, fares badly on the older forms of treatment consisting of inunctions of mercury by the binder or gray powder by mouth.

B. *Acute asymptomatic congenital syphilis* in which the child is well-developed, well-nourished, presents no manifest lesions at birth but yields a persistently positive Wassermann reaction not only within the first two weeks after birth, but beyond this time. I call this type "acute" because the serological reactions indicate an activity of the spirochetes but in tissues not physiologically important and hidden from view, as in the epiphyses of the bones, liver, spleen, etc. This type does very well under vigorous treatment and generally gives a good prognosis.

C. *Late congenital syphilis.*—This group is a very large one and includes those children born apparently healthy and continuing so for several months to several years, when they loose weight, are poorly nourished, develop anemia and secondary infections, epiphyseitis with pseudo-paralyses, cutaneous, osseous or other visceral lesions. The Wassermann reaction is usually positive but may have been negative at birth and is subject to wide variations in the same case at different times according to the fluctuations in spirochetic activity. No other type of syphilis requires more diagnostic acumen and experience than these cases and, indeed, may require the cooperation of several specialists for diagnosis. Sometimes the clinical and serological manifestations are delayed for years and even to adult age. No part of the body is immune to infection and lesions may be found in the eye (especially interstitial keratitis) any time from one year to middle life. In one of my cases the disease was first detected in a physician twenty-six years of age. Neurosyphilis may be detected by spinal fluid examinations in some cases as early as one year of age but clinical manifestations are not usually apparent for five to ten years later.

The specific treatment of these cases is always a prolonged and tedious procedure requiring for success the complete and hearty cooperation of the parents or of the patient, when treatment is undertaken later in life. In many of them, probably the majority, and especially those ranging from five years to adult age, the infection is ground into the tissues as it were, and proves very rebellious, many belonging to the Wassermann-fast group of syphilitics. But many of the lesions disappear under persistent treatment unless they are due to faults in development. Interstitial keratitis, for example, is not as resistant as many text-books state, when adequate intravenous and intramuscular treatment is given. It is true that this and other late lesions are resistant to medication with mercury and iodids by mouth or inunction, but treatment with neoarsphenamin and sulpharsphenamin along with mercury by injection and iodids by ingestion, are doing a great deal more for these patients than has hitherto been the case. It is true, however, that the treatment of this group must be prolonged because the disease is chronic, just as prolonged treatment is required in chronic acquired syphilis. During the first five years the prognosis is greatly influenced by such conditions as digestion and gastro-intestinal disturbances interfering with nutrition, by intercurrent infections and general resistance, etc.; in older children and

adolescents, the prognosis is uniformly better. Among adults, however, there is danger of transmission to the second generation although, in my limited experience, I have encountered but two apparently genuine instances of undoubted second generation infections. Complete and convincing evidence of truly second generation cases is difficult to obtain, satisfactory proof requiring, in the opinion of Higoumenakis,[20] fulfilment of the following conditions: (*a*) The grandchild must present a lesion or dystrophy which is unmistakably syphilitic, and which is not acquired; (*b*) One of the parents must be affected by congenital, but not by acquired, syphilis and the other parent must not have acquired syphilis; (*c*) One of the two parents of the first congenitally affected progenitor—and therefore a grandparent of the affected subject of the second generation—must have acquired syphilis. This author has collected 9 cases reported since 1904 and has added 13 observed by him personally; Riviere[21] has also reported a case. Clinically the lesions and symptoms of second generation syphilis are the same as occur in first generation infections and the treatment is likewise similar.

With modern methods of treatment the prognosis in congenital syphilis is very much better than formerly. Indeed, as stated by Nobl and Remenobsky[22] the prospects of a child surviving infancy are very good and much better than under the older methods of diagnosis and treatment. Syphilitic children appear to withstand the effects of antisyphilitic drugs very well indeed; jaundice, dermatitis and other accidents of arsenical therapy are much less frequent than among adults. On the other hand, complete eradication of the disease is very difficult to the extent of producing a state of persistently negative Wassermann reactions by a sensitive method, even though external lesions may be dissipated. The spirochetes are usually thoroughly disseminated throughout the organs while only a single particular lesion like an interstitial keratitis may be apparent. The great and most important feature is the prevention of congenital syphilis by thorough treatment of potential parents and the treatment of syphilitic women during pregnancy. Of course, faults in development of syphilitic children cannot be corrected even though the disease is held in check or even eradicated. Likewise many of the late lesions are difficult to control or to correct; in other words, some of the injuries produced by *Sp. pallida* in congenital syphilis are no more amenable to correction than in acquired syphilis and especially injuries of the central nervous system. As stated by Morgan[23] the reduction of the mortality alone is not the whole problem in congenital syphilis; in 138 of his cases, 3 per cent. were deaf; 3 per cent. had permanent deformities of the palate, nose, etc.; 8 per cent. had permanent impairment of vision and 12.3 per cent. were mentally deficient. In other words, over 25 per cent. were handicapped in the struggle for existence with permanent physical defects and diminished earning capacity.

Incidence of Congenital Syphilis in Relation to Specific Therapy.—No one can state the incidence of congenital syphilis at the present time, but it is a safe prediction that, under our present methods of treatment, the incidence is decreasing. This is true because we are learning to treat acquired syphilis of men and women much more thoroughly than in former years which reduces the incidence of transmission; furthermore, we are learning the great value of treatment of syphilitic women during pregnancy as an effective means for preventing transmission. The widespread interest in this phase of the modern control of syphilis with the establishment of prenatal clinics, in which syphilis of prospective mothers may be detected and treatment promptly instituted, is alone a great step in advance. Churchhill and Austin[24] have calculated that from an analysis of the literature the incidence of congenital syphilis varies from 2 to 14 per cent. being from 2 to 6 per cent. in our large American cities and 3.3 per cent. in their own experience. Roberts[25] has recently estimated that 10 per cent. of all marriages involves a syphilitic; that 75 per cent. of all offsprings from marriages involving syphil-

itics have syphilis; that 25 per cent. of all fetal deaths are due to syphilis and that 78 per cent. of all syphilitic infants born alive succumb during the first year of life. Jeans[26] states that Wassermann surveys have shown that 9.5 per cent. of adults admitted to hospitals and dispensaries in five of our large American cities, showed positive reactions; that 9.6 per cent. of 5,358 pregnant women in five large cities yielded positive reactions; that the incidence of fetal deaths in syphilitic families have varied according to twelve investigators from different parts of the world, from 17 to 62 per cent. with an average of 30 per cent. while the apparent rate in non-syphilitic families is about 9.7 per cent.; that 30.2 per cent. of infants in syphilitic families die early in contrast with a mortality of 15 per cent. among children of apparently non-syphilitic parentage.

In view of the fact that Wassermann tests and clinical examinations are apt to fail in the detection of many cases of congenital syphilis it is entirely likely that these figures are conservative; at least, the known incidence is alone sufficient for arousing our keenest interest in the subject of the adequate prophylaxis and treatment of syphilis as a means for reducing this tremendous waste of life and health. It may be that the incidence during the next ten years will show little or no change, because, while the true incidence is being reduced by better methods of treatment, more efficient means in diagnosis are likely to be developed with the detection of cases escaping diagnosis at the present time. But I believe it is certain that our present day methods of treatment are reducing the incidence of congenital syphilis and especially by reason of the increasing tendency to investigate the matter of probable syphilis in pregnant women and the prompt institution of treatment when evidences are found; this subject of the prenatal treatment of syphilis may be now discussed with a little more detail.

Prevention of Congenital Syphilis and the Prenatal Treatment of Syphilis. The *prevention* of congenital syphilis refers to such measures as will prevent access of *Sp. pallida* to the child during pregnancy. From the standpoint of the father it means that the parasites must not be present at the time of conception and that he must not infect the mother during the subsequent period of pregnancy. If the *husband* is a syphilitic, a shorough course of treatment should be given in preparation for parenthood, which should be continued as long as sexual relations are held with his wife. If the *wife* is syphilitic, thorough treatment should precede conception and should be continued during pregnancy. If she becomes infected after conception, vigorous antisyphilitic treatment is indicated to prevent, if possible, the access of spirochetes to the fetus; if infection occurs after the eighth month the fetus may be born at term before spirochetemia of the mother and infection of the child occurs, but even though she is infected in the last few weeks of pregnancy, intensive treatment should not be omitted. These, therefore, are measures for the prevention of congenital syphilis.

Prenatal treatment of syphilis strictly refers to the treatment of the fetus through the mother, but may amount to the prevention of syphilis of the child if the treatment of the mother is given early enough to prevent infection. What should be done if the father is syphilitic but the mother presents no clinical or serological evidences of the disease? Is it safe to omit antisyphilitic treatment or should treatment be given? In past decades Fournier, Finger, Gaucher and others believed in the routine treatment of any pregnant wife of a syphilitic man in the interests of the unborn. Carle[27] states that, while he formerly adhered to this practice, he now no longer does so because secret treatment with mercury in pills and syrup is not usually intensive enough anyhow in case the woman is syphilitic and that he prefers to decline

treatment unless the woman shows some evidences of infection, in which case more intensive medication should be given. In my opinion a good deal depends upon the stage of the disease in the father. If more than five years have elapsed since his chancre, with fairly thorough treatment in the meantime, with no clinical evidences of syphilis although the Wassermann reaction is positive, treatment of the mother may be omitted; but if the father's infection is more recent, if his history is uncertain or cannot be ascertained, or, if it is decided to "take no chances" insofar as this is possible without promoting suspicion and discord, I believe it is well to administer antisyphilitic treatment to the mother even though it may be uncalled for and unnecessary in some cases. But what kind of treatment should be given? In the interests of domestic peace and happiness it is frequently advisable to give specific medication without arousing the suspicions of the wife. This of course presents many difficulties. If the father's infection is known to be old and latent it may suffice to give daily inunctions of calomel ointment for a month, followed by bichlorid of mercury and potassium iodid in a syrup by ingestion for a month, followed again by inunctions on alternate months throughout pregnancy. It is frequently possible to give this kind of treatment without exciting suspicion, the inunctions being administered under the disguise of massage.

But if the father's disease is more recent and active, the chances of prenatal infection are much greater and the mother should receive more intensive treatment, to be shortly described. Likewise, if the mother shows a positive Wassermann reaction, regardless of whether or not the father is syphilitic or the stage of his disease, specific treatment is indicated. Stokes[28] believes that practically all women known to have had syphilis should undergo some treatment during pregnancy with due regard for her kidneys, liver and eliminative mechanism in general since these are already under increased burden from the pregnant state. Givan[29] has reported that twenty children of syphilitic women energetically treated during pregnancy yielded negative Wassermann reactions while eleven out of a series of fifteen children of untreated syphilitic mothers, yielded positive reactions. Williams[30] has reported an exceptionally comprehensive study of 96 syphilitic women who had passed through 113 pregnancies, the children being observed for periods ranging from four to twenty-eight months after birth. A particularly valuable feature of his study was the fact that the women were under observation for years enabling him to determine the influence of syphilis on fetal mortality *before* and *after* treatment in the *same* patients. Williams concluded that almost ideal results follow anything like efficient treatment of syphilitic pregnant women and that surprisingly good results may sometimes follow what would ordinarily be regarded as altogether inefficient treatment in men or in nonpregnant women, which would seem to indicate that pregnant women are unusually amenable to antisyphilitic treatment. One woman, however, had received thirty injections of arsphenamin without changing the positive Wassermann reaction, but she gave birth to a child having no evidence of syphilis, and nine months later the child had a negative Wassermann reaction. Williams believes there is something about the pregnant woman which mitigates the virulence of the disease and predisposes to a spontaneous cure. He insists on the early recognition of maternal syphilis so that a healthy child may be born and the mother cured more easily.

Almkvist[31] has recently reported that of 28 untreated syphilitic mothers, 27 bore infants that had manifest signs of syphilis in from 15 days to 8 months after birth; of 39 treated syphilitic mothers, only 4 bore infants with signs of syphilis. He states that more and more the opinion is gaining ground that

for the prevention of congenital syphilis, energetic antisyphilitic treatment is more important during pregnancy than before conception. Ahman[32] states that of 50 syphilitic women treated energetically during pregnancy with arsphenamin and mercury, only 7 or 14 per cent. bore children with signs of syphilis; two of these were infected during the seventh month of pregnancy but received insufficient treatment.

Regarding the kind of treatment to employ, practically all physicians are in agreement on the use of arsphenamin or one of its substitutes in place of mercury. Meyer[33] quotes Sauvage's statement that only 25.46 per cent. living children were born to 217 women with evidences of recent syphilis treated systematically with mercury and iodid, and 10.18 per cent. of the living had signs of syphilis. He then states that salvarsan treatment in seventeen clinics resulted in the birth of 86 to 100 per cent. living children. In his own experience 97.4 per cent. were born alive, 86 per cent. were living by the tenth day and only 15.8 per cent. had a positive Wassermann reaction. Kirkpatrick[34] also states that the organic arsenicals yield best results and that treatment should be instituted as soon as possible but is worth while even in the late months of pregnancy. His method has been to give three courses of eight injections each of neoarsphenamin and of 148 syphilitic mothers so treated, 107 gave birth to apparently healthy children.

A great deal in the success of prenatal treatment depends upon how *early* in pregnancy it is given; certainly the earlier it is instituted the better are the results, probably because infection of the placenta and fetus is prevented. But in cases in which treatment is instituted later in pregnancy, say about the third to fifth months, is it possible to influence the disease in the fetus? We have no accurate information on the question of passage of mercury and the organic arsenicals from the mother to the fetus. Some believe that while the normal placenta is impervious to these drugs that the syphilitic placenta is permeable. Judging largely on the basis of clinical results it would appear that mercury and arsphenamin or one of its substitutes, administered to the mother, reach the fetus with curative effects so that even though the fetus is infected, therapeutic results may follow the administration of antiluetic drugs to the mother.

But may antisyphilitic treatment of the mother be harmful? This important question requires consideration from the standpoint of toxicity of the drugs for mother and child and the possibility of treatment of the mother exciting latent infection in the nature of Herxheimer reactions with added danger of infection of the child. Insofar as toxicity of the drugs is concerned, it would appear that both mercury and the organic arsenicals are well borne by both mother and child. But dosage should be conservative, bearing in mind that the kidneys and liver of the mother are already called upon for extra elimination. Fortunately it would appear, and especially from Williams investigations, that excellent results are to be observed with what may be designated as mild and very conservative treatment because the action of the drugs are probably aided by an increased degree of immunological resistance as a result of the pregnant state. Moderate doses of arsphenamin and neoarsphenamin are well borne and apparently do not promote either abortion or hemorrhage, although I believe preference is to be given to neoarsphenamin and sulpharsphenamin, because these are less vasculotoxic and least likely to produce toxic reactions which may result in abortion or miscarriage and especially since these accidents are always threatened in syphilitic pregnancies.

As regards the possibility of Herxheimer reactions, the subject can only be discussed from the theoretical standpoint. In cases of latent maternal

syphilis in which the fetus may escape infection altogether, it is possible that the administration of antiluetic drugs and especially large doses of the arsenicals by intravenous injection, may excite spirochetic activity with the production of spirochetemia and infection of the placenta and fetus, thereby defeating our very purpose and efforts. For this reason, I believe it is well to institute treatment of these cases with at least one month of mercury and iodids to be followed by the arsenicals; on the other hand, if the mother has acute active syphilis, it is a mistake to give this kind of treatment, since immediate and more energetic measures are indicated.

Fordyce and Rosen[35] have advised a course of 6 to 8 intravenous injections of arsphenamin in dose of 0.3 to 0.4 gm. or of neoarsphenamin in dose of 0.45 to 0.6 gm. at weekly intervals followed by 12 to 15 intramuscular injections of the bichlorid or salicylate of mercury in dose of 1 grain.

Author's Method of Prenatal Treatment.—My method of treatment is usually as follows:

1. If the mother's infection is latent with no evidence of syphilis other than a positive Wassermann reaction, and if treatment is instituted during the first three months of pregnancy, one month of inunctions is first given at the rate of three rubs per week of one dram of blue ointment or 85 per cent. calomel ointment. If inunctions cannot be given, the succinimid or salicylate of mercury in oil are injected intramuscularly once a week, in dose of 1 grain; if these prove too painful, potassium tartrobismuthate in oil is given, once a week, in dose of 0.2 gm. by intramuscular injection.

2. This course is followed by eight intravenous injections of neoarsphenamin in dose of 0.6 gm. once a week or eight epifascial injections of sulpharsphenamin in dose of 0.4 gm. once per week.

3. These two courses require about three months and if pregnancy has now arrived at about term treatment is continued until delivery, in the form of inunctions of mercury or injections of bismuth mercury or mercury by ingestion. On account of the necessity for avoiding gastro-intestinal disturbances and especially diarrhea, I prefer inunctions at the rate of three per week for another month or two; if these cannot be given, either mercury or bismuth by intramuscular injection may be continued at the rate of one every 7 to 10 days but not beyond twelve injections. If pregnancy is about the sixth to seventh month another course of six to eight injections of neoarsphenamin may be given followed by mercury to term.

4. If the mother's infection is early and acute the preliminary month of mercury may be omitted and treatment instituted with injections of neoarsphenamin or sulpharsphenamin in the same manner as described. This course is followed by twelve to fifteen weekly intramuscular injections of either succinimid of mercury in dose of 1 grain or 0.2 gm. potassium tartrobismuthate and after a suitable rest, the course of neoarsphenamin or sulpharsphenamin is repeated, since the indications are to give as intensive and continuous treatment as consistent with safety throughout pregnancy. As so well stated recently by Lawrence,[35a] a great deal may be done toward lowering the incidence of congenital syphilis by the proper treatment of women during pregnancy and especially in prenatal clinics. Underhill and Amatruda[35b] have shown in experiments employing pregnant rabbits and cats that arsenic may be found in small traces in the fetal tissues after the intravenous injection of neoarsphenamin into the maternal circulation and while it apparently did not increase in proportion to the number of injections given, the suggestion is made that the high efficiency of antenatal treatment of syphilis is due to the greater concentration of the spirocheticide in the placenta.

Various Methods for the Specific Treatment of Congenital Syphilis.—Needless to state, many methods have been described for the specific treatment of congenital syphilis but only those of comparatively late years including the administration of the arsenicals, would appear worthy of serious attention, since treatment with mercury and the iodids alone are known to yield inferior results.

Choice of Drugs and Dosage.—Among the organic arsenicals preference is to be given to neoarsphenamin and sulpharsphenamin, the former by intravenous injection. Children appear to stand these compounds very well and it is usually possible to make the doses as large or even slightly larger than given adults per kilogram of body weight. With experience it is frequently surprising how easily and readily intravenous injections may be given to even infants, since neoarsphenamin may be administered with a 1 or 2 c.c. syringe and a fine needle. The doses may be the same for both neoarsphenamin and sulpharsphenamin, namely, about 0.015 gm. per kilogram (2⅕ pounds) by either intravenous or intramuscular injection; arsphenamin is given in slightly smaller dose, namely, 0.010 gm. per kilogram of weight. According to age the doses may be somewhat as follows at weekly intervals:

Age	Neoarsphenamin and Sulpharsphenamin, Gm.	Arsphenamin, Gm.
Birth to 3 weeks	0.04	0.02
4 to 8 weeks	0.06	0.03
2 to 6 months	0.10	0.05
6 to 12 months	0.15	0.10
1 to 2 years	0.20	0.15
3 to 5 years	0.30	0.20
5 to 12 years	0.40	0.30

Children also bear mercury well and it is probably best given by injection; Leredde and Drouet,[36] White and Veeder,[37] Fordyce and Rosen and others have stated that inunctions are inadvisable since it is almost impossible to have them given properly over a long time by mothers; in hospital treatment, however, when inunctions may be given by trained nurses, the method is an excellent means for the administration of mercury. According to Ramsay and Groebner[38] the ordinary 50 per cent. blue ointment is to be preferred to less concentrated forms and need not be repeated more than twice weekly instead of daily. For internal administration mercury with chalk appears to be the favorite; Le Boutillier,[39] however, prefers calomel. The doses of the various mercurials may be somewhat as follows:

A. *By inunction:*

30 to 50 per cent. mercurial ointment: 15 grains twice weekly.
85 per cent. calomel ointment: 15 grains twice weekly.

B. *By intramuscular injection* (bichlorid, succinimid or salicylate in oil; mercurosal or bichlorid in water) once a week:

3 to 8 weeks: $\frac{1}{20}$ grain (0.003 gm.)
2 to 6 months: $\frac{1}{10}$ grain (0.006 gm.)
6 to 12 months: $\frac{1}{8}$ grain (0.0075 gm.)
1 to 3 years: $\frac{1}{6}$ grain (0.01 gm.)
3 to 5 years: $\frac{1}{4}$ grain (0.015 gm.)
5 to 12 years: $\frac{1}{3}$ grain (0.02 gm.)

C. *By ingestion* three times daily:

(1) Mercury with chalk: $\frac{1}{8}$ to $\frac{1}{2}$ grain. Dover's powder may be added: $\frac{1}{20}$ to $\frac{1}{12}$ grain.
(2) Bichlorid of mercury: $\frac{1}{200}$ to $\frac{1}{60}$ grain.
(3) Calomel: $\frac{1}{40}$ to $\frac{1}{10}$ grain.
(4) Potassium iodid: 1 to 5 grains.

Bismuth has been favorably reported upon by Genose and Mazzacuva,[40] Galliott,[41] Bentivoglio[42] and others by intramuscular injection once a week. I have employed potassium tartrobismuthate with success in dose of 0.004 gm. per kilogram for ten to twelve intramuscular injections. For a 6 pound infant this would be about 0.01 gm. or one twentieth the dose ordinarily given an adult at one time, older children receiving larger doses according to body weight.

Mixed treatment with the arsenicals, mercury and iodids is generally employed, very much as in acquired syphilis of adults. Kachel[43] has advocated intr avenous injections of mixtures of neoarsphenamin and mercury (bichlorid, cyanid or novasurol) as described in Chapter LXVII; Engel[44] warns against too intensive treatment of acute syphilitic infants in order to avoid overflooding the tissues with the products of destroyed spirochetes. Indeed, the literature is very full of suggestions, a good review of the subject of congenital syphilis in general having been recently published by White,[45] but it will probably suffice to give here a few of the better known methods developed within the past few years.

One of the best known of the modern methods is that employed by Fordyce and Rosen[46] in the Vanderbilt Clinic of New York. In their method, neoarsphenamin and bichlorid of mercury are given intramuscularly. If the infant is very much under weight with a poor musculature, mercury alone is given at weekly or bi-weekly intervals until there is an improvement in the general condition, after which the injections of neoarsphenamin are begun.

The bichlorid is put up in palmatin in individual collapsible ampules in doses of from $\frac{1}{10}$ to $\frac{1}{8}$ grain or larger for older children. "The object in giving a soluble mercury in oil is to favor slow absorption of the drug. In this form it requires about three or four days for absorption. The palmatin, which is in colloidal state, is semi-solid at room temperature and liquefies at 110°F. When ready for use, the syringe being still warm after its removal from the sterilizer, the contents of the ampule are expressed into the barrel, all the air removed, and if the oil has not liquefied from its contact with the hot syringe, the latter with the drug may be immersed for a minute in hot water.

"An ordinary mercury needle of from 19 to 20 gauge, cut down to from one-half to 1 inch in length, is used. After the buttock has been sterilized with iodin, the injection is given into the muscle about 1 inch from the intergluteal fold near its upper angle. The chief pain is that induced by the prick of the needle, as the children as a rule stop crying as soon as the treatment is completed."

Of 128 treated patients with active syphilis treated by this method,[47] 55 had received two courses or more of neoarsphenamin and mercury intramuscularly; of these 30 patients, or slightly more than 53 per cent., became negative clinically and serologically, the observation period now extending over two years in some instances. The remaining 73 cases consisted of two groups—one made up of younger infants, of whom 22 or 30 per cent., became negative with less than two courses of injections given regularly, and the other made up of older children who had received less than two courses of treatment scattered over irregular intervals and who had remained positive.

Jeans[48] has employed a somewhat similar method and with it White and Veeder report that, of a group of 308 cases whose end results are known, 67 or 22 per cent. were apparently cured or recovered; 108 or 35 per cent. were improved; 55 or 17 per cent. unimproved and 78 or 25 per cent. died.

Gertrude Meyer[49] employs a method consisting of 12 calomel and 12 neoarsphenamin injections over a period of 12 weeks; DeBuys[50] and Schuss-

ler[51] have described methods of mixed treatment with neoarsphenamin and mercury. Adams[52] has employed injections of galyl and gray oil while Crawford[53] and Boone and Weech[54] have employed sulpharsenol (sulpharsphenamin). The latter employed intramuscular injections of 0.02 gm. per kilogram of weight in courses of six at weekly intervals. The patients are then given mercury inunctions for two months in the form of a pea sized portion of one-third strength mercury ointment on a flannel abdominal binder. This course is followed by another course of sulpharsphenamin and treatment continued until three complete courses were given after the first negative Wassermann reaction, so that each child receives a minimum of at least four courses. The local and general reactions to the sulpharsphenamin were found very slight and in 55 per cent. of their patients, the Wassermann reaction became negative after one course of six treatments.

Specific Treatment of Congenital Syphilis after Birth through the Mother's Milk.—As stated in Chapter XLIII, the organic arsenicals, like arsphenamin and neoarsphenamin, are to be found in the milk of many nursing mothers receiving intravenous injections of these compounds. I have seen several cases of newborn syphilitic infants greatly benefited through the milk of mothers under active treatment with these compounds. The administration of mercury to mothers is not followed by as good results. Most writers, however, state that this is not a good or acceptable manner for the treatment of congenital syphilis, but when the arsenicals cannot be given to the child, I believe it is well to give them to the mother if she is nursing her infant, and particularly since she is in need of treatment anyhow if her condition permits.

Author's Method for the Specific Treatment of Acute Congenital Syphilis. I have found it serviceable to divide cases of congenital syphilis into two main groups from the standpoint of specific therapy, namely, (*a*) those presenting evidences of syphilis at birth or soon thereafter and embracing the acute symptomatic and acute asymptomatic types previously described and (*b*) those developing signs and symptoms of syphilis later in life, as after the first year.

The treatment of the first group is, of course, the most difficult because the infection is heavier and more virulent and the child greatly handicapped by poor development, poor musculature and nutrition and a great predisposition to gastro-intestinal disturbances. When they are breast fed by the mother the prognosis is much better; when artificially fed the problem is a difficult one requiring the co-operation of the experienced pediatrician and very careful nursing. Insofar as specific treatment alone is concerned, my usual method is as follows:

1. For the first two months a weekly intramuscular injection of 0.04 to 0.06 gm. of neoarsphenamin or sulpharsphenamin dissolved in 1 to 2 c.c. of sterile distilled water. At the same time two weekly inunctions of *unguentum hydragyrum dil.* (30 per cent.) are given in dose of 15 grains (about 1 gram) gently rubbed into the skin of the abdomen for at least five minutes followed by a flannel binder, which is changed once a week. At the same time the mother should be receiving treatment which may aid the nursing infant in addition to the effects from its own injections.

2. This is followed by a weekly intramuscular injection of bichlorid, succinimid or salicylate of mercury in dose of $\frac{1}{20}$ to $\frac{1}{10}$th grain for three months (12 injections).

3. A rest of one month may now follow or during this period mercury with chalk, with or without Dover's powder, may be given in dose of $\frac{1}{8}$ to $\frac{1}{4}$ grain two or three times a day by ingestion.

4. This is followed by eight more weekly intramuscular injections of neoarsphenamin or sulpharsphenamin without inunctions.

5. This is followed by twelve intramuscular injections of mercury at weekly intervals or six of mercury alternating with six of potassium tartrobismuthate in dose of 0.01 to 0.06 gm. (1/6th to 1 grain) and another month of mercury by mouth.

These courses cover a period of one year and represent the minimum of treatment for any case regardless of serological or clinical results. As a general rule additional treatment is required, and, if so, may be comprised of three of the following courses in the second year and two in the third year: 4 weekly intravenous or intramuscular injections of neoarsphenamin or intramuscular injections of sulpharsphenamin, in dose of 0.2 gm. followed by 8 weekly intramuscular injections of mercury or bismuth; at the same time potassium iodid is given by ingestion in dose of 1 or 2 grains three times a day. Each course requires therefore, about three months and may be repeated with suitable rest periods until satisfactory clinical and serological results are obtained.

Author's Method for the Specific Treatment of Late Congenital Syphilis. Under this heading I refer to cases of congenital syphilis in whom specific treatment is first undertaken after the first year of age and usually between the third and twelfth years, when interstitial keratitis, mental disturbances and other signs and symptoms of syphilis may be first detected along with a positive blood Wassermann reaction. The method is essentially similar to the preceding except that intravenous injections of neoarsphenamin are more readily possible and inunctions of mercury are more satisfactorily administered. For children over twelve years of age, the methods described in preceding chapters for the treatment of late acquired syphilis of adults are applicable; of course, with reduced doses according to age and body weight. This also applies to the matter of intraspinal medication which may be required for children over twelve years but scarcely ever before this time.

1. Treatment is usually begun with three weekly inunctions of mercurial ointment (50 per cent.) or 85 per cent. calomel ointment in dose of 1/3 to 1/2 dram (1 to 3 grams) for a period of one month (12 rubs). At the same time potassium iodid is given by ingestion in daily dose of 4 to 10 grains.

2. This is followed by twelve weekly intravenous injections of neoarsphenamin in dose of 0.2 to 0.3 gm. or twelve weekly intramuscular injections of sulpharsphenamin in dose of 0.2 gm. Potassium iodid is not usually given.

3. This is followed by mercury or bismuth for a period of three months in the form of either (*a*) three weekly inunctions; or (*b*) weekly intramuscular injections of bichlorid, succinimid or salicylate of mercury in dose of 1/6 to 1/4 grain; or (*c*) weekly intramuscular injections of potassium tartrobismuthate in dose of 0.05 gm. (1 grain) or (*d*) alternate intramuscular injections of mercury and bismuth. Potassium iodid is given at the same time in a daily dose of 5 to 20 grains by ingestion (usually 4 grains three times a day).

4. This is followed by a rest of two months without specific medication or during this time mercury with chalk or the protiodid of mercury may be administered. A blood Wassermann test is now made.

Throughout the treatment the urine is examined every 2 to 4 weeks and a careful watch maintained for evidences of toxicity or overdosage of the drugs. Regardless of serological or clinical results, however, treatment is continued for another nine months by repeating the courses described in paragraphs 2, 3 and 4. At the end of this time another blood Wassermann test is made and in the case of older children and particularly those showing clinical signs of neurosyphilis, it is sometimes advisable to examine the spinal fluid. In most cases some clinical or serological evidences of syphilis are still present and specific

treatment must usually be continued for several years longer. For such cases I usually advise two courses of the following per year: 6 weekly intravenous injections of neorsphenamin or 6 weekly intramuscular injections of sulpharsphenamin; or 3 injections of each by alternate injections (the usual plan). These are followed immediately by 6 weekly intramuscular injections of mercury alternating with 6 weekly injections of potassium tartrobismuthate (a total of 12 injections covering 12 weeks). If these injections cannot be given, three inunctions of mercury are given per week for the twelve weeks. At the same time potassium iodid is given by ingestion throughout the course of three months.

Not infrequently syphilitic children of school age are greatly hampered by their disease and it is to be hoped that the Weylander school-hospital plan of management and social rehabilitation will receive the earnest consideration warranted by the importance of the subject and present results.

Prognosis in Congenital Syphilis in Relation to Specific Treatment.—In the preceding pages I have briefly summarized the results being achieved at present in the treatment of congenital syphilis. Great advances have been made within the last ten years but our methods and results are still far from being satisfactory. As a general rule the results in private practice are very much better than in clinic practice because the coöperation of the mothers is so much better and only prolonged, patient treatment yields worth while results. As previously stated congenital syphilis is usually a deeply rooted infection owing to the widespread distribution of the spirochetes and it is even possible that the parasites may have a certain measure of resistance to mercury and the arsenicals in cases in which the mothers have been under treatment. As stated by Fordyce and Rosen, the prognosis depends largely upon: (1) the amount of prenatal treatment; (2) the physical condition at birth; (3) the severity of the infection, whether visceral, cutaneous or neural involvement is present; (4) the time treatment is begun; (5) the type of treatment, and (6) the toleration to treatment. To this I would add, (7) the duration of treatment.

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CHAPTER LXXVIII

SPECIFIC TREATMENT OF SYPHILIS COMPLICATED BY DRUG INTOLERANCE, NEPHRITIS, TUBERCULOSIS AND OTHER DISEASES

As repeatedly stated in preceding chapters, the methods given for the specific treatment of early and late syphilis are in the nature of suggestions only, subject to modification as demanded by individual cases. But I believe they are applicable to the majority of cases, although some conditions like natural or acquired intolerance for our chief antisyphilitic drugs, tuberculosis of the lungs, nephritis, etc., may demand important consideration and complicate any plan for the specific treatment of syphilis. It is my purpose to consider some of these conditions in the present chapter.

Specific Treatment of Syphilitic Individuals Intolerant of the Arsenicals and Mercury.—I use the word *intolerance* to include two classes, namely, those who possess a natural or acquired allergic hypersensitiveness to these drugs and those who bear them well at first and for long periods of time but who gradually become hypersusceptible as a result of accumulative toxic effects. The two conditions are usually separate entities but sometimes sharp lines of differentiation cannot be drawn, although *intolerance* expresses the clinical result in either case.

Fortunately allergic hypersensitiveness to arsphenamin and its substitutes and mercury are quite rare, that is, the type of case who cannot tolerate the drugs at all in therapeutic amounts. This subject is considered with more detail in Chapters XLVII and LI; up to the present time, I have not

encountered anyone with an intolerance for bismuth, nor have I met with individuals who were intolerant for both arsenic and mercury at the same time. Furthermore, intolerance for a drug may be largely a matter of method of administration; this is especially true of mercury which may be poorly borne in one form or by a certain method and route of administration while satisfactorily tolerated in another form or route of administration.

But in exceptional cases of syphilis and especially of late neurosyphilis, a peculiar intolerance may be manifested for arsphenamin and neoarsphenamin administered by intravenous injection in that violent immediate and early reactions or such late accidents as dermatitis, preclude a series of injections of adequate amounts. It is highly probably that sulpharsphenamin by intramuscular injection may be better tolerated in such cases because of the slower absorption, but this compound has not been in use long enough to warrant a definite statement at the present time, except to mention that Stokes and Behny have employed it in cases who developed toxic reactions to arsphenamin without toxic effects. In other words, an intolerance may be present for a particular compound like arsphenamin while neoarsphenamin is well borne; or for neoarsphenamin when sulpharsphenamin is well borne, etc.

Fortunately, intolerance for the organic arsenicals is quite rare in early syphilis and it is in these cases that intensive arsenical therapy is mostly required. As previously stated it is unwise to give less than 0.1 gm. of these compounds to adult patients with early syphilis even at close intervals since animal experiments have shown that in acute syphilis such small doses may stimulate rather than destroy spirochetes, although in chronic syphilis and especially chronic cardio-vascular syphilis, small doses over long periods of time may constitute a valuable method of treatment.

I have, however, treated cases of early syphilis with 0.1 gm. doses of arsphenamin and neoarsphenamin by intravenous injection every day for the first five days, followed by two to three injections per week for a total of twenty-five injections as a course, followed by mercury, etc., as outlined in Chapter LXXII. This dose was tolerated with occasional attacks of nausea and diarrhea when 0.3 gm. and larger doses produced severe reactions. It is probable that epifascial or intramuscular injections of sulpharsphenamin, 0.1 to 0.2 gm. in each buttock every five days, may be given to cases of this kind, but I have had no experience with this compound in such up to the time of this writing and would refuse to use it altogether in cases of post arsenical dermatittis.

In cases of extreme arsenic hypersensitiveness it may be necessary to abandon any plan of treatment with arsphenamin and its substitutes altogether and depend upon mercury or bismuth. The latter is probably to be preferred on account of its superior spirocheticidal properties and may be substituted for the arsenicals by giving potassium tartrobismuthate, sodium and potassium tartrobismuthate, bismuth trioxid, or some other suitable compound by intramuscular injection every five to seven days in dose of 0.1 to 0.2 gm.until a total of about 2.5 gms. have been injected. Intravenous injections would be still better in early syphilis on account of more rapid effects, but are regarded as quite dangerous from the standpoint of toxicity. The injections may be followed by mercury as described, but, since both metals involve the gums and gastro-intestinal tract in a similar manner, great care must be exercised in regard to toxic reactions.

If bismuth is not given it may be well to consider intravenous injections of mercury and for this purpose flumerin in dose of 0.2 to 0.3 gm. or mercurosal in dose of 0.1 gm., every five days for six injections as a course, are worthy of consideration and to be preferred to bichlorid of mercury and other soluble mercurials. These may be followed by mercurial inunctions but great care is

required in regard to toxic reactions, especially stomatitis, and it is well to watch the kidneys carefully for evidences of irritation by frequent examinations of the urine for albumin and red blood corpuscles.

Sometimes it is possible in these cases to give an occasional small injection of neoarsphenamin even though some nausea, vomiting and looseness of the bowels may follow; if so, this practice is very desirable, as better effects are produced than in treatment alone with either mercury or bismuth.

Fournier's Chronic Intermittent Treatment of Syphilis.—Even though injections can not be given, largely because of cowardice on the part of the patient—and I have known a physician contracting genital syphilis who absolutely refused injections of the arsenicals and mercury and who died five years after infection with cerebral syphilis—it is still possible to treat by Fournier's method and Fournier apparently cured some cases of acute early syphilis. His plan was somewhat as follows, subject to variation for individual cases:

1. Four courses of mercury by mouth in the first year. Protoiodid was generally given in dose of 0.1 gm. daily. This corresponds to about 1½ grains in divided doses, like ¼ grain six times daily. Each course is of two months duration with periods of one month without specific medication.

2. During the second year, three courses are given, and two or three in the third year.

3. In the fourth year he gave three or four courses of mercury and potassium iodid each of four to six weeks duration; the average dose of potassium iodid was forty-five grains daily either along with the mercury or alternating with mercury if necessary.

4. In the fifth year three courses of mercury and potassium iodid were given, each of four to six weeks duration, and two courses in the sixth year.

Specific Treatment of Syphilis Complicated by Nephritis and Hepatitis.—The treatment of syphilis occurring in individuals with acute or chronic nephritis or disease of the liver and gall tracts, presents unusual difficulties on account of the possibility of toxic reactions from interference with elimination of the drugs. In early syphilis and, especially during the secondary stage, both acute nephritis and acute hepatitis may be caused by the *Sp. pallida* with prompt improvement of the lesions upon the institution of specific medication, as already discussed in Chapter LXXV.

In both syphilitic and non-syphilitic nephritis, I believe that it is safe to administer the arsenicals in preference to either mercury or bismuth, but arsphenamin (0.2 to 0.4 gm.) is probably better than neoarsphenamin (0.4 to 0.6 gm.) because the former is really less renotoxic. As stated in Chapter LXXV, Munk,[1] Hoffmann,[2] Audrey,[3] Stokes[4] and Cole[5] have preferred arsphenamin for the treatment of acute syphilitic nephritis and I believe it is always to be preferred in these as well as other types of presumably non-syphilitic nephritis. But it is sometimes advisable to first conduct the phenolsulphonephthalein test for elimination and to gauge doses and arrange treatment accordingly. Of course the elimination of this substance may not be a true index of the functional capacity for eliminating arsenic, mercury or bismuth, but, according to my experience, there is but little trouble and danger in treatment when the test shows a total elimination in two hours of at least two thirds the average normal return.

However, the dose of the chosen drug must be reduced below that usually employed and the intervals of injection should be at least 5 to 7 days. Treatment, therefore, is milder but more prolonged with careful watch for evidences of toxic reactions and especially various skin manifestations. If intravenous injections cannot be given, as may be the case in children, epifascial injections of sulpharsphenamin may be administered and this method is always to be

preferred in the presence of large amounts of albumin and casts in the urine because of the slower absorption throwing less burden upon the kidneys than intravenous injections. After giving courses of arsenic, it is better to follow with intramuscular injections of bismuth in dose of 0.1 gm. every 5 to 7 days than with injections of mercury, because the former is less renotoxic. If these are refused, mercury may be cautiously given by inunction, but I am absolutely opposed to intramuscular injections of the salicylate, calomel or gray oil on account of having seen several disastrous cases of explosive stomatitis from cumulative effects. If mercury is injected, it is far better to inject the soluble succinimid in almond oil in dose of 1 grain or mercurosal in dose of 0.05 (1 grain) in saline once a week. Potassium iodid is generally well borne.

In the presence of infection or other disease of the liver and gall tracts, preference is to be given intravenous injections of neoarsphenamin (0.4 to 0.6 gm.) or intramuscular injections of sulpharsphenamin, as both are less hepatotoxic than arsphenamin. It may be well in cases of this kind to apply tests for hepatic efficiency as the bilirubin content of the blood or the phenoltetrachlorphthalein test mentioned in Chapter LI. The significance of these in relation to the administration of anti-syphilitic drugs has not yet been established but I believe it may be at least stated that when the results approximate normal and usual values that we may precede with treatment more boldly than when the results indicate a severer degree of hepatic injury. I frequently apply one or both of these tests to icteric or sub-icteric individuals with syphilis, acute or chronic, before outlining the plan of therapeutic attack. But it is well under any circumstances to make the doses smaller than usual and the intervals not less than five days. Courses of either arsphenamin or sulpharsphenamin may be followed by mercury or bismuth as described, as either drug is less dangerous than in cases of severe nephritis.

Specific Treatment of Syphilis Complicated by Tuberculosis and Leprosy. Tuberculosis bears an important relation to syphilis both because the latter appears to favor infection with the tubercle bacillus and antisyphilitic drugs may activate or otherwise influence tuberculous lesions in an unfavorable manner. Ravogli[6] states that in many cases tuberculosis is associated with syphilis and that tuberculosis which was latent during good health may, in consequence of syphilitic infection, become active and destructive. Marino and Fournier,[7] and Vedder[8] also assert that syphilis predisposes to tuberculosis and Hollander and Narr,[9] in a recent review of the literature covering 6324 cases of tuberculosis, assert that 494 or 10.36 per cent. were positively syphilitic.

Owing to the effects of arsenic upon capillaries, it is well not to employ intravenous injections of arsphenamin or neoarsphenamin in the treatment of early syphilis occurring in cases of tuberculosis of the lungs with hemorrhages. Intramuscular injections of sulpharsphenamin are probably much less dangerous, but I have had no experience with the drug in such cases. If there are no hemorrhages, intravenous injections may be given, but neoarsphenamin is very much to be preferred to arsphenamin, not only because it is less vasodilating, but also because concentrated solutions may be injected with a minimum of excitement and disturbance and without raising blood pressure.

The questions frequently arise: How do mercury and neoarsphenamin act upon the organism in the presence of clinically demonstrable tuberculous foci, and how do patients having latent tuberculosis tolerate energetic treatment with these preparations? Does this treatment favor the progress of an already manifest tuberculosis, and does it arouse closed latent foci to renewed activity? In a study by Teiche,[10] syphilis alone was never found to create

a state favorable to the distribution of tuberculosis, but the tuberculosis invariably manifested itself in the course of the treatment, although not usually until after a very energetic course of treatment. In patients having primary syphilis, who were subjected to intensive treatment, the body can hardly be damaged by syphilis, as the bulk of the spirochetes are destroyed before they can do harm. Nevertheless, an existing pulmonary tuberculosis may become worse, or a latent tuberculosis become active, suggesting that the principal danger may be referable to incautious treatment, which creates favorable conditions for the distribution of the tuberculosis. When a disposition to tuberculosis is explained by an impairment of the biologic chemical adjustment of the body against the tubercle bacillus, it is readily understood that the two disturbing components such as mercury and arsenicals, may occasionally upset the complicated system of forces which still maintain the balance so that the fight is decided in favor of the tubercle bacilli. This radical chemical therapeutic procedure perhaps temporarily interferes with the production of antibodies, thereby opening a wide entrance avenue to the distribution of the tubercle bacilli. But Teiche does not mean to exaggerate the danger of combined mercury and arsphenamin treatment in patients having latent tuberculosis since many tuberculous individuals readily tolerate such combined treatments. Under no circumstances, however, should a cure of syphilis with intensive treatment be enforced at the expense of the tuberculosis. How much may be ventured depends principally upon the toleration of the specific preparations and the character of the pulmonary or other form of tuberculosis. It should always be remembered that the syphilitic infection is still accessible to later treatment, whereas tuberculosis, when it has once become progressive, has an extremely unfavorable prognosis. A series of observers, with whom Teiche is obliged to agree, regard a recent syphilitic infection as less responsible for the progressiveness of an existing tuberculosis than the institution of antisyphilitic treatment.

In less severe cases the treatment may be along the lines described above but always with smaller doses than given ordinarily and with at least five to seven day intervals. Mercury and bismuth are ordinarily well borne, but iodids should be given only with caution, although Madrid[11] states that they do not prove injurious. Hartz,[12] Elliott[13] and others state that arsphenamin or its substitutes in small doses at long intervals appear to be the drugs of choice and that mercury should be used with great care, since its deleterious effects may not be immediate, but appear after several months. Potter[14] has expressed a similar opinion.

The indications for iodids in the treatment of *acute syphilis* are only slight—indeed I believe that they can be dispensed with altogether, since syphilitic fibrosis has not occurred, and iodid tends to soften tuberculous shells of fibrous tissue and to thereby spread tuberculous infection; Hollander and Narr have ascribed the malignancy of tuberculosis in one of their syphilitic patients at least in part to the iodids, whicht hey were forced to give because of an acute nephritis preventing the administration of both neoarsphenamin and mercury.

In tuberculosis of the skin, however, arsphenamin is ordinarily well borne and indeed, may have a beneficial influence as reported by Stokes and others (Chapter VIII).

The iodids are generally regarded as contraindicated in *leprosy* by most experts in this disease, when the coexistence of syphilis demands antisyphilitic treatment. Otherwise the leper appears to bear well both the organic arsenicals and mercury although the doses and route of administration require careful supervision to meet the special conditions of individual cases.

Specific Treatment of Syphilis Complicated by Diabetes Mellitus.—As stated in Chapter LXXV, in a discussion of the specific treatment of syphilis of the pancreas, the relationship of syphilis to diabetes is not clear, although it is well substantiated that the pancreas may be infected with *Sp. pallida* with evidences of diabetes and that when syphilis and diabetes coexist, specific treatment is not generally followed by improvement of the diabetes. However, Paullin and Bowcock[15] have recently reviewed the literature covering the reports of cases of diabetes apparently due to syphilis and improving under antisyphilitic treatment, adding a case of their own of glycosuria, but not classified as one of diabetes mellitus, in which antisyphilitic treatment was followed by an apparent cure of the glycosuria.

Diabetes does not usually interfere with the usual treatment of early or late syphilis if the patient is in good nutritional state and the glycosuria under control. In the presence of severe diabetes with emaciation, etc., considerable care in specific medication is required. In my experience intramuscular injections are frequently followed by excessive local reactions, potassium tartrobismuthate in oil being better tolerated than either sulpharsphenamin or the mercurials. I believe that specific treatment should consist mainly of large doses of the iodids by ingestion and mercury by inunction; small doses of neoarsphenamin (0.4 to 0.6 gm.) may be given intravenously and if the inunctions cannot be given, potassium tartrobismuthate in dose of 0.1 to 0.2 gm. by intramuscular injection may be used as a substitute.

Specific Treatment of Syphilis Complicated by Cardiovascular Disease and Focal Infections.—In advanced cardiovascular disease with decompensation or threatened decompensation of apparently non-syphilitic origin and especially that due to mitral lesions, the organic arsenicals may be given, but for intravenous injections neoarsphenamin is very much to be preferred to arsphenamin because least likely to produce disturbances of blood pressure and the vasomotor system. It is very probable that intramuscular injections of sulpharsphenamin will prove particularly useful in these cases. Mercury is to be preferred to bismuth, partly because its diuretic effects are of aid in cases of decompensation. Of course, if syphilitic infection of the myocardium or aorta are present, and this is always quite probable, treatment may be modified as suggested on page 978 for the specific treatment of cardiovascular syphilis.

In chronic *focal infections* and especially severe gingivitis with apical abscesses and infected tonsils, due care must be exercised in view of the possibility of injections of arsphenamin and its substitutes lighting up such foci with the production of dermatitis, stomatitis and allied reactions. Neoarsphenamin is the drug of choice among the arsenicals and bismuth is to be preferred to mercury.

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CHAPTER LXXIX

CRITERIA OF CURE AND THE PROGNOSIS OF SYPHILIS IN RELATION TO SPECIFIC THERAPY

The criteria of cure and the prognosis of syphilis have been already discussed from different angles in preceding chapters and especially in relation to the specific treatment of different clinical types, but it may not be amiss to attempt the presentation of a brief résumé of these subjects in view of their great importance. The task is by no means an easy one as physicians undertaking the treatment of syphilis well appreciate when an honest and sincere attempt is made to answer the never failing question: "Can I be cured?" The pathologist may be too pessimistic in his reply; the clinician, mistaking clinical and serological improvement for complete cure, may be too optimistic. The patient, frequently weighed down by worry from a feeling of moral guilt and the dread of paresis, tabes dorsalis and such consequences, may easily become a victim of the deepest pessimism with the creation of those mental or pschyic disturbances so difficult of cure and aptly designated by Collins as the "syphilitic scars of the spirit;" or, as a result of prompt eradication of obvious lesions and symptoms, hastily develope a state of excessive optimism with consequent neglect of advice regarding future treatment and observation. The physician is not infrequently to blame for either state and should endeavor to maintain a midway attitude which I believe he truly may, when equipped with a good working knowledge of the pathology and immunity of disease and the possibilities of thorough modern treatment. Altogether too frequently one encounters individuals with the late lesions of syphilis, long neglected, because of the assurance previously given by physicians that complete cure had been obtained; also patients in good health, by all criteria, in the depths of gloom and despair, because of the statement that "once syphilis always syphilis."

What Constitutes the Cure of Syphilis?—Upon first thought this question may sound foolish and unnecessary since the answer may be: when all of the spirochetes have been eradicated from the tissues. This is the only reply that the pathologist can give; but may it not be unduly exacting from the clinician's point of view? Is there such a thing as the "cure" of syphilis in the clinical sense even when we are reasonably sure that some living spirochetes still remain in the tissues? The serologist is apt to insist upon a state of permanently negative Wassermann reactions with the blood and the absence of all abnormalities in the cerebrospinal fluid. But is it possible to have a "cure" in the clinical sense with persistently positive blood Wassermann reactions and various spinal fluid changes?

So the question of the "cure" of syphilis is subject to wide variations in interpretation and must be determined in each case by thorough investigation from different angles. Some physicians are willing to base an opinion solely upon the clinical status; this is a mistake and especially since the accuracy of clinical observation and detection of syphilitic lesions is subject to even greater variation than the much abused Wassermann reaction.

Others are willing to base an opinion largely upon the results of sero-biological tests and these may lead to error, because there is a limit to their sensitiveness as well as wide variations in the data they yield according to the methods employed. If we had a test so sensitive and so specific that it could detect the very last vestiges of syphilitic infection, our search for a sign or signs of the cure of syphilis would be ended; but we do not have such and there is therefore, much room for speculation and opinion, neither of which are lacking in the literature upon the treatment of syphilis. As stated above, it is an easy matter to answer the question on what constitutes the cure of syphilis by stating, the complete eradication of all living spirochetes and the correction of the damage they have done. But how do we know, during life at least, when all spirochetes are eradicated? And is it possible to correct the damage they have done even if this is accomplished? Or should we be content to put out the fire even though unable to rebuild the house? "Cure" from the standpoint of most patients means the complete eradication of all spirochetes, complete correction of all damage and total restoration of all impaired functions; this is their hope and ideal. But the physician knows that, while these ends may be accomplished in some cases and especially in early cases of syphilis efficiently treated, the word "cure" has to be interpreted a little less exactly in the majority of cases and made to include not a few in which all further progression of the disease is stopped for the usual span of years even though the pathologist may find after death the presence of unhealed lesions apparently producing no signs or symptoms during life.

Criteria of the Cure of Syphilis from the Clinical Standpoint.—The clinical criteria of the cure of syphilis may be summarized as follows:

(1) The complete and total absence of all signs and symptoms of *progressive* visceral infection. This, of course, cannot be determined by a single examination but only by very careful physical examinations at intervals over a period of years.

(2) The improvement or regression of lesions as the result of healing. In this connection is to be remembered, however, that some damage cannot be repaired and especially injuries of the central nervous system. While we may confidently expect some improvement in most cases, yet if further progression is stopped, all is being done that may be expected. Indeed, a nerve cell or tract injured by *Sp. pallida* may continue to degenerate by reason of nutritional disturbances even after the organisms have been destroyed and the same may be true in the liver, heart and other organs. Furthermore, the very act of healing may introduce a new set of symptoms as shown by the development of ascites due to the contraction of fibrous tissues in a syphilitic liver. Likewise in the aorta or other artery, healed patches of mesarteritis with fibrosis may stretch under pressure and result in dilatation or aneurysm even though the syphilitic process itself primarily responsible for the condition has been checked or even eradicated. In syphilitic aneurysms of the aorta it is not uncommon to find that stretching is progressive when the Wassermann reaction indicates a great reduction in spirochetic activity and I have known of cases to succumb with syphilitic apoplexies when there were reasons for believing that the syphilitic processes were completely checked, as indicated by progressive reduction in the Wassermann reactions during life and the results of histo-pathological examination after death. In paradoxical cases of this kind, it is very easy indeed, to fall into the error of ascribing such progressive changes to active syphilis whereas they may be due to processes of healing leaving weak links in a chain that breaks under functional activity. I freely grant that the distinction cannot always be made clinically, but the possibility of such paradoxical results must be kept in mind.

(3) In some cases roentgenological examinations are of value as showing cessation of syphilitic changes, especially in the aorta, and they should be enlisted as additional clinical evidence of cure.

(4) Mental symptoms must not develop or, if present and due to syphilis, should not become progressively worse. This, however, is a subject that I am not able to discuss at length because a differentiation must be made between those psychic disturbances due to syphilis and those of other origin. Only a skilled psychiatrist can pass judgment upon these and especially one trained in the special psychic phenomena of syphilis. But it may be stated that if psychic changes due to organic lesions of syphilitic origin are present, that they too must show clinical improvement if "clinical cure" is claimed to have been accomplished.

It is ordinarily stated that following the treatment of early syphilis, one of the criteria of cure is freedom from all manifestation for at least a year; it is obvious that this time is too short since internal lesions may develop and easily escape detection. The period of observation must be over a number of years with occasional physical and serological examinations, although specific medication need not be given during this time.

Criteria of the Cure of Syphilis from the Sero-biological Standpoint.—The sero-biological criteria of the cure of syphilis may be summarized as follows:

(1) A persistently negative blood Wassermann reaction. A single negative reaction is of little or no significance in this relation, because if specific medication is stopped upon its appearance, positive reactions occur at later periods in many cases. As a criterion of cure, the test should be repeated every four months for at least two years and then once or twice a year thereafter for an additional several years. The technic must be as sensitive as consistent with specificity and methods lacking in sensitiveness are capable of doing more harm than good because of the mis-information and sense of false security which they yield. Is a persistently negative blood Wassermann reaction to be accepted as a good criterion of cure? May it not be negative in the presence of active lesions? My answers to these important questions may be now based upon six years of experience with my new complement fixation method, which I believe combines the maximum of sensitiveness consistent with specificity; the experiences of others may not agree with mine because the technic employed for conducting "Wassermann tests" varies so much in different laboratories and exerts such a tremendous influence upon the results in latent syphilis that falsely negative reactions may occur when the numbers and activity of the spirochetes are so reduced that sufficient "reagin" or antibody is not present in the blood serum to yield a positive reaction unless a sensitive method is employed. But falsely negative reactions are extremely rare in the presence of active lesions of syphilis. Stokes and Des Brisay report about 7 per cent. falsely negative reactions in active cases of syphilis but my experience in no way corresponds to theirs. In neurosyphilis the blood reaction may be negative when the spinal fluid shows positive reactions, but this is largely confined to asymptomatic cases. My experience teaches me that the positive complement-fixation reaction is one of the last signs of syphilis to disappear under treatment; that it is one of the first to recur if "cure" has been incomplete; that it may be a sign of spirochetic activity when there are absolutely no clinical signs or symptoms of syphilis; that a persistently positive reaction by a technically correct and acceptable method is a sure sign of syphilis, despite a condition of good general health and the begetting of apparently healthy progeny; that an individual yielding a persistently positive reaction by an acceptable method cannot be regarded as "cured" in the complete sense although he may be in the clinical sense inso-

far as symptoms are concerned; that a persistently negative reaction is not an infallible index of complete cure although, when based upon a sensitive and technically correct method, it is one of the safest and best criteria of "cure;" that the majority of individuals yielding persistently negative reactions by a sensitive method need not fear the development of late lesions and especially if this state is associated with a normal spinal fluid.

(2) A provocative Wassermann test with negative results is of additional value if conducted along the lines laid down in Chapter XLI, but as a general rule is of much less value as a criterion of cure than as an aid in diagnosis of untreated cases of latent syphilis.

(3) Persistently negative flocculation reactions and especially by Kahn's method, have much the same significance as a criterion of cure as persistently negative complement fixation reactions. In occasional cases of treated syphilis, however, the flocculation reaction may be positive when the complement fixation reaction is negative; the reverse occurs less frequently. I do not know at this time what may be the proper interpretation of these reactions, since more time and experience are required for evaluation, but I believe it is safe to assume at present that they may be an indication of continued infection and that only persistently negative reactions in both tests are acceptable as a criterion of "cure."

(4) The spinal fluid must show no increase in pressure; the cells must not be above 10 per cubic millimeter; there must be no increase in proteins or reduction in dextrose; the colloidal gold, colloidal mastic, colloidal benzoin and similar tests must yield negative reactions and the complement fixation test must yield a negative reaction with a sensitive method employing large amounts of fluid. Is one examination of the spinal fluid with such results sufficient? If made after the first six months or year of treatment, it probably is in the great majority of cases but a second examination two or three years later is advisable in order to be surer that there is no involvement of the central nervous system and this is especially true, if abnormalities were found in the first examination. Can neurosyphilis be present with a perfectly normal spinal fluid? In other words, is a normal spinal fluid a guarantee that the meninges, brain and cord are absolutely free of syphilis? I believe it is in the majority of cases, but that small vascular lesions and even very early posterior root and ganglion meningitis of incipient tabes dorsalis may escape detection and especially by one examination (much less so by repeated examinations). A negative spinal fluid examination therefore, is not an absolute criterion of freedom from syphilitic infection, but apparently, in the great majority of cases, is acceptable as an indication of freedom of infection of the central nervous system and especially when repeatedly negative.

Criteria of the Cure of Syphilis from the Pathological Standpoint.—These criteria are of course not available during life but are of great value for aiding us in a proper estimation of the clinical and sero-biological criteria of cure. They may be summarized as follows:

(1) There must be no gross lesions of syphilis in the aorta, heart, liver, meninges and brain, bones, joints, etc. However, when the treatment of syphilis was begun after the development of lesions in the tissues, a distinction should be made if possible between the fibrosis of healing and the fibrosis of syphilis. The lymph-glands, for example, may remain fibrotic apparently after all spirochetes have been removed. Fibrotic patches in the aorta, liver, etc., may be sterile; we greatly need more investigation along these lines by rabbit inoculation tests and histological examination for *Sp. pallida* to determine whether these lesions are still syphilitic or the healed or healing lesions of syphilis in the absence of *Sp. pallida*. In other words, the mere finding of a gross lesion or lesions cannot be accepted as a sign of incured syphilis unless

they are evidently progressive and inflammatory. Should we insist upon finding the spirochetes before labeling the lesions as syphilitic? Not if I may judge by the frequency with which prolonged search of large numbers of serial sections have yielded negative results in my experience. But may the lesions of syphilis be diagnosed with certainty by naked eye examination? Some may and especially the aortic lesions, but microscopical examinations are usually required before a lesion or lesions may be definitely diagnosed as syphilitic. Can the presence of syphilis in a cadaver be excluded by naked eye examination alone? By no means and this brings up the second criterion:

(2) There must be no histological evidences of syphilis in the organs. But is it possible to identify syphilitic lesions with certainty by histological tissue changes? In other words, is there a typical tissue change in syphilis? According to my experience, both questions must be answered negatively. When *Sp. pallida* is found, the answer is in the affirmative, although other spirochetes may be mistaken for *Sp. pallida* in the tissues, but are not likely to be encountered if frambesia tropica, infectious jaundice and other spirochetic infections can be excluded. I am not at all sure that the incidence of syphilis in cadavers is as high as Warthin states and great care must be exercised against the error of mistaking histological changes due to other types of tissue injury for syphilitic lesions, including those that may have been caused by mercury and the organic arsenicals employed in treatment. Upon first thought we may conclude that thorough macroscopical and microscopical examination of the tissues after death of subjects known to have had syphilis would furnish an absolute criterion of the question of cure, but such is not the case when the above and additional sources of error in both a positive and negative way are kept in mind. There is no one criterion or method for settling the question; just as the clinician and serologist may err, so may the pathologist, and the latter by no means may claim the distinction of being the final arbiter of the question.

Is Syphilis Curable?—So, with all these criteria and demands of evidence regarding the question of the cure of syphilis, we may well ask, Is the disease curable?

In the first place is syphilis curable without the use of drugs, that is, by immunological processes unaided by specific medication? Undoubtedly many untreated cases of syphilis develop long periods of clinical and serological latency approaching in these respects as good a state of general health, working capacity and efficiency as our best treated cases, but, as stated in Chapter XXXVII, there are no reasons for believing that the body defenses are capable of completely ridding the tissues of *Sp. pallida* unaided by specific medication in any more than a very small, doubtful and unknown percentage of cases. It is my opinion, therefore, that the complete and spontaneous cure of syphilis is of exceptional occurrence and that any syphilitic who deliberately refuses treatment and decides to take a "chance," is running grave risks of developing the late lesions of the disease. Negroes appear to have a better chance in this direction than whites and women of both races, who have borne children, have a much better chance than the males of both sexes, since syphilitic pregnancies appear to enhance the immunological mechanism of resistance to *Sp. pallida.* It is true that the Wassermann test may become persistently negative in untreated cases of syphilis, but this is not alone acceptable as a criterion of cure or safety and especially since many of such cases show the spinal fluid changes of asymptomatic neurosyphilis. But immunological or tissue resistance is sufficient in many cases for holding *Sp. pallida* in check and in the cases of syphilitics who have reached 55 to 60 years of age, who are in good general health and apparently symptom free because of extreme latency of infection even though the Wassermann reaction is positive, I believe it is well to depend

upon a continuation of this state to carry the individuals to the usual span of life, rather than to institute active specific medication which may disturb the latency and activate lesions beyond the means of our control, much less our ability to cure.

Is syphilis curable by specific medication? I do not hesitate to state as my opinion that many, the majority, of cases of early syphilis treated according to the modern intensive methods, are apparently cured by all the criteria applicable during life. I state "apparently" because no one is able at present to express a definite and final opinion since sufficient time has not yet elapsed for judging the results of modern therapy. But how about those cases treated by the old mercury-iodid method of Fournier, Hutchinson and others? We now know that these methods did not cure as frequently as the older syphilologists believed, since they depended entirely upon clinical criteria while we have brought to bear upon the question the much more exacting sero-biological methods. But no one can deny that many cases of syphilis were apparently cured by these older methods as judged by all of our present day criteria. In other words, I believe it may be stated that early syphilis is curable in many instances by the older methods of treatment and a much larger percentage by modern methods, if one may express a preliminary opinion of the latter by results up to the present time. On the other hand, there are some cases of syphilis apparently incurable by even the most persistent hammering with all the drugs in the therapeutic arsenal for syphilis. Virulence of strain, possibly the question of tissue affinity of the strain, natural resistance and above all, inherited or acquired predisposition to infection of the central nervous and cardiovascular systems, intolerance for drugs, persistence in treatment, etc., are factors of varying importance that influence the results in any given case.

Is chronic or late syphilis curable? I believe that many cases are to the extent of being rendered free of symptoms and serologically negative over the usual span of years, even though absolute or complete "cure" may not have been attained, as determined by the finding of microscopical evidences of the disease after death. But after all "clinical cure" is the most important consideration. In the majority of cases of late syphilis efficient treatment is at least capable of arresting the progress of the disease and relieving many of the symptoms, even though cure in the sense of complete eradication of all spirochetes may not be possible. Indeed, it may not be advisable or wise to even attempt securing the latter result if there are chances of injuring the liver, kidneys and other organs by excessive amounts of antisyphilitic drugs, or if the administration of these reduces the general health of the individual by their toxic or untoward effects.

Just as we can never be sure of the complete cure of tuberculosis, arthritis and other chronic bacterial infections, neither can we be sure of the cure of syphilis and especially chronic or late syphilis; but we can render the individual free of danger from infection to others including the progeny and bring about a state of clinical and sero-biological cure that so nearly approaches complete eradication of all spirochetes as to amount practically to the same thing. But before we are safe in pronouncing any one as "cured" the following conditions are demanded:

Clinical criteria
- (1) Complete absence of all signs and symptoms of active syphilis over a period of years although the individual may show the permanent effects of the disease after progression has ceased and healing occurred.
- (2) The regression and healing of lesions.
- (3) Roentgenological evidences of regression and healing.
- (4) Absence of syphilitic psychoses.

Sero-biological criteria	(1) A persistently negative Wassermann reaction by a sensitive method over a period of years. (2) A negative provocative Wassermann reaction. (3) Persistently negative flocculation reactions over a period of years. (4) Persistently negative spinal fluid reactions.
Pathological tissue criteria	(1) No macroscopical lesions of active syphilis although healed lesions may be present. (2) No microscopical or histological evidences of syphilis, although healed lesions may be present. (3) Absence of *Sp. pallida* in the tissues.

Relation of the Kind of Treatment to the Cure and Prognosis of Syphilis. To attain these ends it is furthermore quite surely indicated that specific treatment must be prolonged, persistent and sufficiently intensive. With the discovery of arsphenamin it was hoped that a short and royal road to cure was possible, but we now know that the syphilitic should undergo almost as long as a course of treatment and observation as advised by Fournier in the pre-arsphenamin days.

The influence of the kind of treatment or no treatment at all, upon the incidence of lesions, latency, or apparent recoveries, has been very well brought out by Stokes and Des Brisay.[1] They observed that spontaneous arrest of the disease, symptomatic and serologic, occurred in less than 1 per cent. of 208 untreated cases. Following treatment by mouth 6 per cent. were totally arrested and 10 per cent. were serologically negative on blood and spinal fluid after more intensive treatment. Among other conclusions reached by these investigators, bearing upon the present subject and with which I agree, may be mentioned the following:

"Cases treated by modern methods even though inadequate, exhibit a definitely lower proportion of cardiovascular, visceral, osseous, cutaneous and mucosal complications than untreated or mouth-treated cases.

"The proportion of serologically and symptomatically negative cases is much greater when even inadequate modern treatment is given than in untreated cases.

"Medical treatment of syphilis is absolutely necessary to reinforce the defense mechanism in the aggregate control of the disease.

"The decision not to treat a patient with demonstrable syphilis, implies reliance on his defense mechanism and should only be reached after the most thorough going appraisal of the status of that mechanism. Mere appearance of health or the result of any single test is not sufficient basis for decision."

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